AN ADVANCED LABORATORY MANUAL OF ORGANIC CHEMISTRY

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BOOK DEPARTMENT

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PREFACE.

In the field of organic chemistry there are a number of elementary laboratory manuals, any one of which may be used to the student's advantage. When it comes to the choice of a guide for an advanced course, however, there is a vast amount of material available from which a selection in the form of a laboratory manual has never been made. Hence the student is often permitted to follow some line in which he is interested, regardless of its practicability or its value from the standpoint of training, or else the planning of the experiments devolves entirely upon the instructor.

With the object of providing a brief advanced course in manipulative organic chemistry embodying experiments scattered as widely as possible over the important types of substances and reactions, the author desires to present this little book in the hope of rendering simpler the task both of the advanced student and his instructor.

It has been the writer's aim to select experiments of greater difficulty than those ordinarily included in elementary manuals, but to avoid preparations of so difficult or involved a nature as to become a source of discouragement rather than a stimulus to the student. In this connection a word of apology may be necessary for including as much as has been done of the work of Dr. Walter A. Jacobs of the Rockefeller Institute and the writer, but it is very strongly felt that the value of a volume such as the present one depends largely on the personal experience of its author, and for this rea-

son the writer has drawn freely on his own work an
that of his colleagues. It has been attempted also t
preserve as just a balance as possible between the chen
istry of aliphatic and aromatic compounds, and to in
clude products of technical and biological, as well a
theoretical importance, in order to provide as broad
foundation as possible for the student in his futu
work. In the selection of experiments, care has bee
taken to exclude those involving great expense, and
further economy is effected by the use of many of th
initial products as steps in the synthesis of others.

Finally, the author takes great pleasure in acknow
edging his indebtedness to his colleagues at the Rock
feller Institute for Medical Research for the use o
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this manual

MICHAEL HEIDELBERGER.

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INTRODUCTORY WARNING.

The student will remember from his elementary course that many organic reactions, harmless under controlled conditions, may gather speed and violence if not carefully watched. Every experiment should therefore be considered as a whole from the point of view of its potential sources of danger, and a plan of procedure mapped out accordingly.

If the reaction is accompanied by a rise of temperature, even if no minimum is specified in the directions, accidents may often be prevented by keeping a pot of ice water or freezing mixture at hand, into which the vessel may be plunged in time to prevent boiling over or decomposition. If gases such as hydrobromic acid, for example, are evolved, the reaction should be carried out under the hood, and the vapors led into a flask of water by a tube terminating above the surface.

Many a weary repetition may also be avoided by keeping in pots as much as possible large flasks or beakers containing material on which much time has been expended.

It should also be kept in mind that most organic compounds are more or less toxic and many are extremely dangerous. Distillations other than under diminished pressure should therefore be carried out under the hood, and care should be taken to avoid contact of the substances handled with the skin, or inhalation of their vapors or dusts. The writer still has vivid

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recollections of several extremely uncomfortable days many years ago as a result of getting minute traces of ω-tetrabromo-*o*-xylene, , on his finger tips and thence indirectly on his face and into his eyes. When working therefore with substances which are known to be irritating the student will find it advisable to wear rubber gloves. Dried crystalline material or powders should also be transferred under the hood, a precaution which it is particularly unsafe to overlook in the case of the alkaloid, arsenic, and mercury derivatives of which the preparation is described in the following pages.

The student will also frequently handle highly inflammable solvents, and must therefore remember that many painful and even fatal accidents have resulted from working with these near a flame or electric switch.

While there need be no occasion for timidity, it must be borne in mind that constant vigilance and concentration are the price that must be paid for the joys, the satisfaction, and the thrills that come to those who work in organic chemistry.

AN ADVANCED LABORATORY MANUAL OF ORGANIC CHEMISTRY

I. NITRATION AND NITROSATION.

(*See also p. 92*)

A. Nitration.

***o*-Nitraniline, o-$O_2NC_6H_4NH_2$.**

In the nitration of benzene, it will be remembered, only one mononitro compound was capable of formation, and the by-product occurring in the reaction was *m*-dinitrobenzene:

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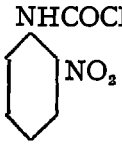
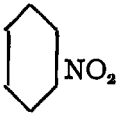
If one substituent is already present in the benzene nucleus, however, the case becomes more complicated, and using aniline or its acetyl derivative as an example, three isomeric mononitro derivatives are theoretically possible:



o-Nitro-acetanilide.



m-Nitro-acetanilide.

p-Nitro-acetanilide.

The relative amounts of the position isomers formed vary within wide limits according to the conditions used, for if one nitrates aniline itself in cold, concentrated sulfuric acid a relatively large proportion of *meta-* and *para-* nitranilines are the chief products, while if acetanilide is nitrated in the same solvent the *para-* nitro derivative is obtained almost exclusively. If the nitration is carried out in an excess of *fuming* nitric acid the main product is again the *para-* compound, with only 6 to 8 per cent of *o*-nitraniline. Witt and Utermann [1] found, however, that by carrying out the nitration in glacial acetic acid in the presence of acetic anhydride as dehydrating agent, about 75 per cent of the acetanilide nitrated was converted into the *ortho-* compound, the remainder being *p*-nitro-acetanilide. By this method large amounts of *o*-nitran-

[1] *Ber.* 39, 3901 (1906), 41, 3090 (1908).

iline can readily be prepared. Batches of the size given below may be conveniently handled in the laboratory.

Nitration.

90 g. of acetanilide are dissolved by warming gently in a mixture of 80 g of acetic anhydride and 44 g. of glacial acetic acid, and the solution is then chilled in ice-water. 50 g. of fuming nitric acid (d 1.52) are mixed with 46 g. of glacial acetic acid, also cooled, and added in small portions to the chilled acetanilide solution, from which a portion of the acetanilide may separate. The mixture is stirred well with a thermometer, adding the nitric acid solution slowly enough to prevent the temperature from rising above that of the room, and controlling the speed of the reaction by immersing in ice-water when necessary. When the tendency of the temperature to rise becomes very slight the reaction mixture should be removed from the ice-water and allowed to stand 24 hours at room temperature. Care should be taken by cooling occasionally if necessary during the first hour or two that the reaction does not become too vigorous. The next day the mixture is poured on to ice, stirred well, and the crude crystalline *o*-nitro-acetanilide filtered off, washed well with ice-cold water, and sucked as dry as possible.

Separation of Isomers.

In the meantime a mixture of one volume of 50 per cent aqueous potassium hydroxide, 4 volumes of water, and one volume of alcohol is prepared, cooled to 0°, and the nitration product thoroughly rubbed up (in portions) in a chilled mortar with about 600 cc. of the solution. The *o*-nitro-acetanilide dissolves, while the *para*- compound remains insoluble in the cold mixture and is sucked off and washed with a little of the cold

solution, then with a little ice-cold water. After recrystallization from water the yield is 20 g, melting at 207°.

Saponification.

The filtrate and washings from the crude *para*- nitro derivative are now allowed to come to room temperature, and on letting stand for 24 hours saponification occurs and pure *o*-nitraniline separates in long, orange red needles, the reaction being as follows:

$$o\text{-}CH_3CONHC_6H_4NO_2 + H_2O \longrightarrow CH_3COOH + o\text{-}H_2NC_6H_4NO_2,$$

just as acetamide, CH_3CONH_2, when warmed with dilute alkali, splits into acetic acid and ammonia. After washing with ice-cold water the yield of *o*-nitraniline is 30–40 g., melting at 71.5°.

B. Nitrosation.

p-Nitroso-*o*-cresol,

While the nitrosation of phenols occurs at least as readily as that of tertiary aromatic amines such as dimethylaniline, the conditions must be very carefully controlled owing to the ease with which most phenols oxidize. For this reason a higher proportion of tarry by-products is formed and the yields are smaller than in the case of the dialkylanilines. Taking *o*-cresol as a typical example of a phenol with an unsubstituted *para*- position, the main product of the reaction is *p*-nitroso-*o*-cresol.

54 g. of *o*-cresol are dissolved in 4 liters of ice-water in a battery jar provided with an adequate mechanical stirrer, and 34 5 g of 100 per cent sodium nitrite (or an equivalent amount of a less pure salt) are then added. Since nitrosation is effected by means of free nitrous acid and not its salts no reaction takes place at this point. A solution of 18.5 cc. of concentrated sulfuric acid in 500 cc. of water is then added through a dropping funnel during one-half to three-quarters of an hour, keeping the temperature between 5° and 10° by means of additional ice, and stirring continually. In this way the nitrous acid reacts with the cresol as fast as liberated to yield the *p*-nitroso compound, and since any local excess of nitrous acid is largely avoided by the slow addition of the sulfuric acid and the efficient stirring, the formation of tarry by-products is reduced to a minimum While the nitroso compound may separate oily at first, it soon crystallizes. After standing in the cold for one to two hours after the addition of the sulfuric acid the mixture is filtered on a large Buchner funnel and washed with ice-cold water. The resulting brown solid is purified by dissolving in 10 per cent sodium carbonate solution, stirring with bone-black to collect insoluble tar, and filtering into an excess of dilute sulfuric acid. 40–45 g. of the nitroso compound should be obtained in this way as glistening, orange-brown scales melting at 134°.

II. HALOGENATION.

A. Chlorination.

Chloroacetone, $ClCH_2COCH_3$

If acetone be treated with a chlorinating agent such as phosphorus pentachloride, the keto group is attacked and 2,2-dichloro-propane, $CH_3CCl_2CH_3$, results. If, however, elementary chlorine is used, the hydrogen atoms of the methyl groups are successively replaced. Not only is a mixture of mono- and poly-chlorinated acetones formed, but the hydrochloric acid liberated condenses the acetone to products of higher molecular weight, of which mesityl oxide may be taken as an example.

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CO + H_3CCOCH_3 \longrightarrow \begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!C.CHCOCH_3.$$

Thus, unless some substance is at hand to bind the hydrochloric acid as fast as formed, exceedingly complex mixtures are obtained from which it is virtually impossible to isolate pure products; mesityl oxide, for instance, boiling at practically the same point as monochloroacetone. Fritsch [1] found that small pieces of marble were very satisfactory, as these reacted at once with the hydrochloric acid liberated, and his method is accordingly the basis of that given below.

[1] *Ann* 279, 313 (1894).

Chlorination.

21 g. of marble, broken into small pieces, and 84 g. of acetone are placed in a flask provided with an inlet tube, dropping funnel, and reflux condenser, and warmed to 40° in a water bath. A slow stream of chlorine is then passed in and enough water (a total of 50 to 60 cc. slowly dripped in to keep in solution the calcium chloride formed by interaction of the marble and hydrochloric acid This is also aided by frequent agitation of the flask. The reaction must be very carefully watched, for if a yellow color develops (and according to Kling[2] this usually happens at the lower reaction temperature originally given by Fritsch) it indicates the formation of hypochlorous acid, and this, if it accumulates, may react explosively with the acetone In the event, then, that the solution turns yellow the stream of chlorine is at once interrupted until the coloration disappears. When only a little marble is left, the reaction is discontinued, for although a large excess of acetone is present the main product would be the symmetrical dichloro derivative, $ClCH_2COCH_2Cl$, if this excess were not maintained. The mixture is allowed to stand at 40° until the evolution of carbon dioxide ceases, making sure that an excess of marble is present, and is then poured off from the marble into a separatory funnel. The two layers formed are separated and the lower, consisting of a strong aqueous solution of calcium chloride, is discarded.

Fractionation.

The upper layer of acetone and its chlorination products is fractionated with the aid of a good distilling column, the monochloroacetone boiling at 118-20°. The yield is 16.8 g., plus an additional 5 g. on refractionation of the lower and upper fractions.

[2] *Bull. soc chim.* [3] 33, 322 (1905).

The chloro-acetones are extremely irritating, both in vapor form and if dropped on the skin. Gloves should be worn and all operations conducted under the hood. This applies to the next experiment as well.

B. Bromination.

dl-α-Bromopropionic Acid, $CH_3CHBrCO_2H$.

The method used depends upon the fact that although acetic acid and its homologs react with difficulty with bromine, the anhydrides and acid bromides readily yield bromo substitution products.[8]

Acid Bromide.

To 50 g. of propionic acid and 7.6 g. of dry amorphous phosphorus are added, drop by drop, 66.7 g. of bromine, at about which point evolution of hydrobromic acid ceases. Through the intermediate formation of phosphorus bromides the acid is converted into the bromide according to the equation (Zelinsky):

$$4CH_3CH_2CO_2H + P + 5Br \longrightarrow 4CH_3CH_2COBr + PO(OH)_3 + HBr.$$

Bromo Acid Bromide.

In order now that substitution should take place it is unnecessary to isolate the acid bromide—the mixture is simply warmed to 40-50° on the water bath, using a reflux condenser, and an additional 100 g. of bromine is added drop by drop, the reaction being:

$$CH_3CH_2COBr + Br_2 \longrightarrow CH_3CHBrCOBr + HBr.$$

Bromination proceeds rapidly and may be considered complete two hours after all of the bromine has been added. Bromopropionyl bromide boils at 154° under

[8] Hell, *Ber.* 14, 891 (1881); Volhard, *Ann* 242, 141 (1887); Zelinsky, *Ber.* 20, 2026 (1887); Wenig, *Ann.* 280, 247 (1894).

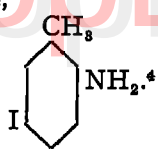
atmospheric pressure, but distillation of the mixture is then difficult, and it is accordingly purified by distillation in a moderate vacuum. The yield is 75-80 per cent of the theory.

Bromo Acid.

If an ester of α-bromopropionic acid were desired the appropriate alcohol would now be used, but as the acid itself is required, the bromide is decomposed by addition of one and one-third equivalents of water, the mixture being shaken under a reflux condenser, with a pot of ice close at hand, until homogeneous, and finally warmed for one-half hour on the water-bath. The solution is then cooled, treated with several volumes of ether, dried over sodium sulfate, and concentrated. When fractionated *in vacuo* the residue boils mainly at 124° under a pressure of 18–19 mm. and solidifies in a freezing mixture, then melting at about 25°. The acid should be protected from the moisture of the air, as it is quite hygroscopic. It is also a powerful skin irritant. The yield should be 60 per cent of the bromide used.

C. Iodination.

5-Iodo-2-toluidine,



In general the introduction of iodine into the aromatic nucleus is not as readily effected as in the case of chlorine or bromine, but aromatic amines unsubstituted in the *para*- position nevertheless react easily with iodine.

[4] Wheeler and Liddle, *Am. Chem. J.* 42, 501 (1909).

An equimolecular amount of iodine is dissolved in *o*-toluidine, substitution taking place with evolution of heat and conversion of one-half of the base into the hydriodide, according to the equation:

$$2CH_3C_6H_4NH_2 + I_2 \longrightarrow I(CH_3)C_6H_3NH_2 + CH_3C_6H_4NH_2.HI.$$

In order to utilize the remainder of the *o*-toluidine and iodine the reaction is completed by heating the mixture under a reflux condenser with an equal volume of water, 2 molecular equivalents of powdered calcium carbonate, and 2 volumes of ether to take up the iodo base formed, the entire reaction being represented by

$$2CH_3C_6H_4NH_2 + 2I_2 + CaCO_3 \longrightarrow 2I(CH_3)C_6H_3NH_2 + CaI_2 + CO_2 + H_2O.$$

After one hour's heating the ether is allowed to boil off and the mixture is distilled with steam, the iodo compound passing over slowly in a yield of 75 per cent of the theory. The practically pure product is dried and recrystallized from ligroin, forming prisms which melt at 90–1°.

III. SUBSTITUTIONS.

A. β-Chloropropionic Acid.

1. **Ethylene Cyanohydrin**, $HOCH_2CH_2CN$.[1]

32 g of ethylene chlorohydrin, $ClCH_2CH_2OH$, are dissolved in 160 cc of absolute alcohol and boiled under a reflux condenser To the boiling liquid is added, drop by drop, a solution of 27.2 g. of potassium cyanide in 42 cc of water, and the boiling continued for 8–10 hours. A precipitate of potassium chloride forms, and at the end this is filtered off, washed with a little alcohol, and the filtrate concentrated to a syrup and fractionated *in vacuo*. The yield of cyanohydrin should be 20 g., boiling at 110° under 15 mm pressure.

2. **β-Chloropropionic Acid**, $ClCH_2CH_2CO_2H$.

10 g. of ethylene cyanohydrin are heated in sealed tubes at 100° with 75 cc. of concentrated hydrochloric acid for three hours If the cyanohydrin is boiled with dilute sodium hydroxide[2] or warmed with acid in dilute alcohol,[3] hydrolysis of the nitrile or CN group occurs,

$$HOCH_2CH_2CN + 3H_2O \longrightarrow HOCH_2CH_2COOH + NH_4OH,$$

and the so-called hydracrylic acid is formed, among other products. If, on the other hand, the cyanohydrin

[1] Moureu, *Bull. soc chim* [3] 9, 426 (1893); Jacobs and Heidelberger, *J. Am. Chem Soc* 39, 1465 (1917).
[2] Wislicenus, *Ann* 128, 6 (1863)
[3] Erlenmeyer, *Ann.* 191, 268 (1878).

is heated in a sealed tube with concentrated hydrochloric acid, not only is the nitrile group saponified to carboxyl, but chlorine replaces the hydroxyl group,[4] the end result being

$$HOCH_2CH_2CN + 2HCl + H_2O \longrightarrow ClCH_2CH_2COOH + NH_4Cl.$$

There is no evidence at hand as to whether the intermediate product is hydracrylic acid or $ClCH_2CH_2CN$, or both, although it is known that β-chloropropionic acid can be prepared from the former.[5]

The contents of the tubes are diluted with just enough water to dissolve the ammonium chloride which has separated and extracted repeatedly with ether, thorough extraction being necessary, as the chloropropionic acid is also quite soluble in water. After drying the ethereal extracts over anhydrous sodium sulfate and concentrating, a syrupy residue is left, which crystallizes readily on cooling and rubbing with a rod The yield should be 10.5 g. Recrystallized from ligroin, it melts at 38.5–9 5° (corr.).

Similarly 10 g. of ethylene cyanohydrin, boiled three hours with 100 cc. of hydrobromic acid (d 1.49) and worked up in the same way, yield 17 g. of β-bromopropionic acid, melting at 60–1° (corr.) after recrystallization from ligroin.

B. Benzylamine, $C_6H_5CH_2NH_2$.

Benzylhexamethylenetetraminium Chloride.

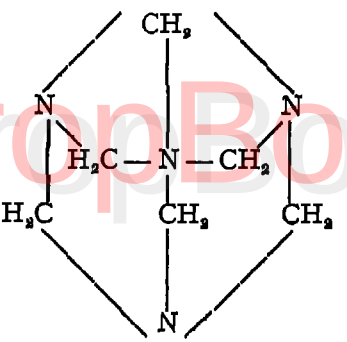
70 g. of benzyl chloride are added to a suspension of 70 g. of finely powdered hexamethylenetetramine in 4 parts of chloroform, heated to boiling under a reflux condenser on the water bath, and removed if necessary

[4] Jacobs and Heidelberger, *loc. cit.*
[5] Beckurts and Otto, *Ber.* **18**, 226 (1885).

until the initially often vigorous reaction is over. The mixture is then heated one-half hour longer, placing the flask on a cork or rubber ring to protect it from the bumping which usually occurs

This method, of course, is an indirect one for replacing the halogen of benzyl chloride by ammonia. If ammonia itself is used, the usual mixture of primary, secondary, and tertiary bases is formed, and the yield of primary benzylamine is poor. Delépine [6] found, however, that the primary amine was the main product if benzyl chloride was first combined with hexamethylenetetramine and the resulting compound suitably decomposed As the method is of wide application for the preparation of primary amines it is given here.

As is well known, formaldehyde and ammonia combine to yield hexamethylenetetramine, $C_6H_{12}N_4$. Duden and Scharff [7] found that most of the reactions of this substance could be explained on the basis of the three dimensional formula.



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Among its other properties, hexamethylenetetramine combines with one molecular equivalent of an aliphatic

[6] Delépine, *Bull soc. chim.* [3] 17, 293 (1897).
[7] Duden and Scharff, *Ann.* 288, 218 (1895).

halide, RX, such as methyl iodide or benzyl chloride, to yield quaternary ammonium salts of the type $C_6H_{12}N_4.RX$,[8] just as trimethylamine forms quaternary salts of the type $(CH_3)_3N.RX$. The chief objection to the Duden and Scharff formula is that the reaction stops when *one* equivalent of halide has reacted, while according to the formula, which postulates four tertiary nitrogen atoms, four molecules of halide should combine with one of hexamethylenetetramine. However, only one enters into reaction, and for our present purpose the equation is:

$$C_6H_5CH_2Cl + C_6H_{12}N_4 \longrightarrow C_6H_{12}N_4 < \begin{matrix} CH_2C_6H_5 \\ Cl \end{matrix}$$

Isolation of the Salt.

The reaction mixture is cooled, filtered, and the salt washed with a little chloroform and sucked dry The yield should be 90 per cent of the theory. The salt darkens above 180° and melts at 192°

If some of the salt is dissolved in a little water and boiled, formaldehyde is evolved, and the solution suddenly becomes turbid, owing to the decomposition of the quaternary salt to yield methylene-benzylamine, $C_6H_5CH_2N\ CH_2$, and as this product is fairly stable, vigorous treatment is necessary for its hydrolysis, as will be seen below.

Decomposition.

The salt is transferred to a distilling flask provided with a condenser and treated with the theoretical amounts of 95 per cent alcohol and concentrated hydrochloric acid according to the equation:

$$C_6H_{12}N_4.C_6H_5CH_2Cl + 3HCl + 12C_2H_5OH \longrightarrow 6CH_2(OC_2H_5)_2 + 3NH_4Cl + C_6H_5CH_2NH_2.HCl,$$

[8] An indication of the extent to which RX may be varied may be obtained by glancing through *J Biol Chem*, 20, 659, 685; 21, 103, 145, 439, 455, 465 (1915).

the formaldehyde split off combining at once with the alcohol to form "methylal." The flask is rotated gently until the salt is dissolved and then warmed carefully until crystals of ammonium chloride begin to separate, removing the source of heat until certain that the reaction does not become violent. The liquid separates into two layers, of which the upper is mainly methylal. When this no longer increases it is distilled off, and the residue in the flask cooled and filtered. The mixture of ammonium chloride and benzylamine hydrochloride on the filter is washed with some of the hydrochloric acid-alcohol mixture, the filtrate returned to the flask, and treated with one-third of the original amount of acid-alcohol mixture. A volume of liquid equal to that added is now distilled off, and the process again repeated, by which time the distillate should be free from methylal. As stated above, a methylene compound is initially formed, the equation being:

$$C_6H_{12}N_4.C_6H_5CH_2Cl + 3HCl + 10C_2H_5OH \longrightarrow 5CH_2(OC_2H_5)_2 + 3NH_4Cl + C_6H_5CH_2N{:}CH_2.HCl,$$

and the prolonged treatment described is necessary in order to decompose this completely.

Benzylamine.

The final residue in the flask and the crystals already filtered off are dissolved in water, chilled, and the solution made alkaline with strong sodium hydroxide solution or solid sodium carbonate. The amine layer is separated, dried over a few sticks of sodium hydroxide, and distilled.

A representative run started with 70 g. of hexamethylenetetramine and 70 g. of benzyl chloride gave 129 g. of the quaternary salt, which in turn yielded 45 g. of pure benzylamine, boiling at 184°.

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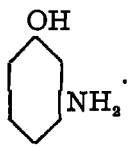
C. ***m*-Aminophenol,** 

.

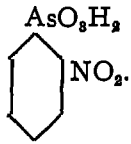
Of the methods for the preparation of this substance two would seem best suited for laboratory use In the first place, one can start with *m*-nitraniline, diazotize this, and decompose the diazo compound by boiling with dilute acid, forming *m*-nitrophenol, but as the yield is not very good and the nitrophenol must subsequently be reduced to the amino compound, the method is scarcely suited for the preparation of considerable amounts of this material. On the other hand, the method involving the direct replacement of one of the hydroxyl groups of resorcinol, m-$C_6H_4(OH)_2$ by ammonia, was found to be very satisfactory.

The direct replacement of a phenolic hydroxyl group by the amino group is relatively difficult in the benzene series, requiring high temperatures, although it takes place somewhat more readily in the case of the naphthols. If, for example, resorcinol and ammonia alone are heated under pressure, a high reaction temperature is required, and the yield of *m*-aminophenol is poor. If, however, ammonium chloride is present,[9] the reaction may be carried out at a lower temperature and the yield is more satisfactory.

200 g. of resorcinol, 120 g. of ammonium chloride, and 400 cc of 10 per cent aqueous ammonia are heated in an autoclave in a bath the temperature of which is 220°, the heating being continued for 14 hours after the pressure reaches a constant value The yields are smaller if the heating is carried out at a lower temperature or for a shorter period and are not improved

[9] Ger pat 49,060.

by longer heating. After the autoclave and contents have cooled, the mixture is concentrated to dryness *in vacuo*, taken up in 650–750 cc. of hot water, and allowed to cool. 90–100 g. of crude *m*-aminophenol separate on standing in the cold. The filtrate is acidified strongly with concentrated hydrochloric acid and shaken out several times with ether to remove unchanged resorcinol. The aqueous liquor is then treated with an excess of ammonia and again shaken out with ether, an additional 30–35 g. of aminophenol being recovered. The crude product is recrystallized from 2–3 parts of water, about 115 g. separating as sandy crystals melting at 123°.

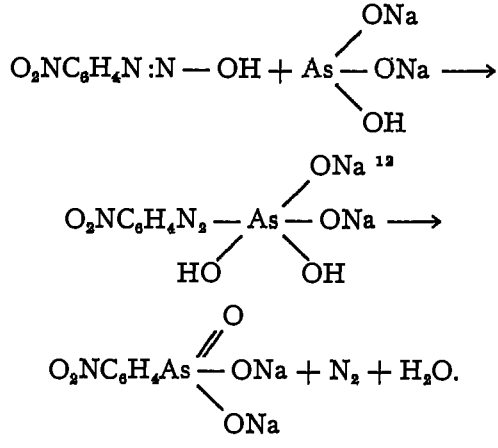
D. ***o*-Nitrophenylarsonic Acid,** 

The student is already familiar with the replacement of the aromatic amino group by the cyano- and halogen residues by means of the Sandmeyer reaction, and its conversion into the phenol group through the diazo reaction has been touched upon in the discussion accompanying the preceding preparation. With these examples the usefulness of the diazo group as an intermediary between the amino and other groups is by no means exhausted, and the so-called Bart reaction,[10] given here, affords a typical example of the extensive application of the fundamental reaction in question. One of the examples in Bart's patent is *o*-nitrophenylarsonic acid, but his description of the substance is inaccurate and the variation of the method here given is believed to be more convenient.[11]

[10] Ger pat 250,264.

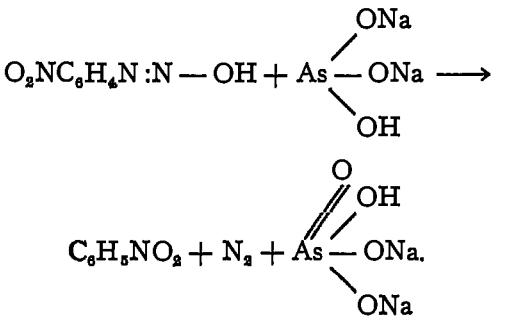
[11] Jacobs, Heidelberger and Rolf, *J. Am. Chem. Soc* 40, 1582 (1918)

50 g. of *o*-nitraniline (p. 13) are ground finely under 250 cc. of 1 : 1 hydrochloric acid, and the mixture is chilled to 10° and slowly diazotized with a strong solution of 27.5 g. of sodium nitrite. After 10 to 15 minutes' stirring the solution is filtered from traces of undissolved nitraniline and poured slowly with vigorous rotation into 275 cc. of thoroughly chilled 25 per cent sodium hydroxide solution, keeping the temperature below 0° by immersion in a freezing mixture. The resulting alkaline solution is added to a cold solution of 67.5 g. of sodium arsenite in 625 cc. of water, using a sufficiently large flask to allow for the frothing which occurs during the subsequent decomposition of the diazo compound. The mixture is then heated on the water bath to 60–70° for 1.5 to 2 hours, during which a slow but steady nitrogen evolution occurs. Overheating is particularly to be avoided. The reaction may be considered to take place according to the following equation:



[12] Cf. Schmidt, *Ann* 421, 159 (1920).

Owing to the fact that sodium arsenite is a reducing agent, the reaction does not proceed quantitatively, some of the diazo compound being reduced with formation of nitrobenzene:



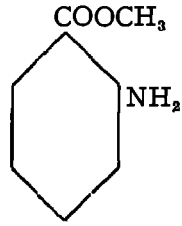
Purification.

After the evolution of nitrogen ceases the hot mixture is made faintly acid with acetic acid, shaken with boneblack to remove the tarry by-products, and filtered. *o*-Nitrophenylarsonic acid is a strong acid, and is not liberated by a slight excess of acetic acid, so that in order to obtain the free acid the deep yellow filtrate must be treated with hydrochloric or sulfuric acid until strongly acid to Congo red. After chilling thoroughly and letting stand 55 g. of *o*-nitrophenylarsonic acid are obtained as a heavy, pale yellow powder. Recrystallized from water it forms pale yellow, glistening, hexagonal plates containing one molecule of water of crystallization. After this is driven off *in vacuo* at 100° the acid melts and decomposes at 235–40°, with preliminary softening.[18]

[18] Instead of Expt. V B the nitro acid may be reduced with ferrous sulfate and alkali according to *J. Am. Chem. Soc.* **40**, 1583 (1918), yielding *o*-arsanilic acid.

IV. ESTERIFICATION, ETHERIFICATION, DE-ALKYLATION, AND RELATED REACTIONS.

A. Esterification; Preparation of the Perfume, Methyl Anthranilate.[1]



Either anthranilic acid itself, or acetanthranilic acid, the intermediate product in the preparation of anthranilic acid from *o*-acet-toluidide, may be used for the esterification, since alcoholic hydrochloric acid hydrolyzes, or rather alcoholyzes, the acetamino group.

7 g of anthranilic acid or 9 g. of the acetyl derivative are dissolved or suspended in 50 cc of methyl alcohol which has previously been dried over potassium carbonate. Dry hydrochloric acid is passed in until the solution, which becomes hot, is saturated. It is then boiled for one hour under a reflux condenser surmounted by a calcium chloride tube. When the solution is cooled methyl anthranilate hydrochloride crys-

[1] Cf Erdmann, Ger. pats 110,386, 113,942.

tallizes. The mixture is diluted with about 200 cc. of water and made alkaline with sodium carbonate. The oily ester is shaken out with ether and the ethereal solution washed first with 5 per cent sodium carbonate solution, and finally with water. The ethereal layer is dried over sodium sulfate and evaporated to small bulk, after which the ester is distilled *in vacuo*. It boils at 135° under a pressure of 15 mm of mercury, and forms a crystalline mass when cooled and rubbed, melting at 24.5°.

Methyl anthranilate occurs in oil of orange blossoms (neroli oil), oil of orange peel, and in the essential oil of jasmine flowers, and is extensively used in perfumery.

B. *p*-Nitrophenoxyacetic Acid,

The reaction of phenols with alkyl halides or dimethyl sulfate in the presence of alkali to combine with the acid liberated is, of course, well known. The following preparation [2] is selected as an example of the variety of alkyl compounds that enter into this reaction, it being, of course, possible to consider chloroacetic acid, $ClCH_2CO_2H$, as a methyl halide in which one of the hydrogens has been replaced by a carboxyl group.

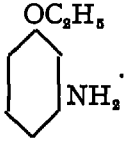
35 g. of *p*-nitrophenol, 40 g. of 50 per cent sodium hydroxide solution (the additional molecular equivalent being to neutralize the chloroacetic acid), 24 g. of chloroacetic acid, and 200 cc. of water are boiled gently

[2] Jacobs and Heidelberger, *J. Am Chem. Soc.* 39, 1437 (1917).

in an open flask until the solution is no longer alkaline to litmus. As the etherification is not quantitative owing to the action of free hydroxyl ions on the chloroacetic acid according to the equation:

$$ClCH_2COONa + OH^- \longrightarrow HOCH_2COONa + Cl^-,$$

one-half of the above quantities of alkali and chloroacetic acid and 50 cc. of water are added, and the solution is boiled again until neutral. It is then acidified strongly with hydrochloric acid and cooled, precipitating the nitrophenoxyacetic acid The crude product may be purified either by recrystallization from alcohol, or by dissolving it in dilute sodium hydroxide solution and reprecipitating with hydrochloric acid. The yield should be 25–30 g. The acid forms glistening platelets which melt at 183°, as recorded in the literature

C. ***m*-Phenetidine,** 

Acetylation.

m-Aminophenol may be conveniently acetylated by dissolving it in a little more than the minimum amount of warm 50 per cent acetic acid, cooling quickly to room temperature, and adding 1.1 molecular equivalents of acetic anhydride. After one-half hour the solution is diluted with an equal volume of water and chilled, rubbing the walls of the vessel to complete the crystallization. The product so obtained is filtered off, washed with ice water, and dried. It should melt at 148-9° [3] and is then sufficiently pure for preparative purposes.

[3] Ikuta, *Am. Chem. J.* 15, 41 (1893)

Ethylation.

27 g. of *m*-acetaminophenol are dissolved in 180 cc. of normal potassium hydroxide solution, warmed to 50-60°, and shaken with 23 cc of diethyl sulfate,[4] added in small portions, keeping the temperature within the limits indicated. It is of course necessary to protect the amino group, otherwise this, too, will be alkylated, and acetylation is a very convenient method for this purpose, as the acetylamino group is unaffected by the conditions used in the reaction, and may be readily removed by saponification at a later stage. The temperature must be raised owing to the fact that diethyl sulfate is more inert than dimethyl sulfate, with which methylations can be carried out in the cold. It is thus also less sensitive than dimethyl sulfate to the hydroxyl ions present in the alkaline reaction mixture, and good yields of ethyl ethers may therefore be obtained with its aid in spite of the saponification of the reagent into alcohol and sulfuric acid, which does occur to an appreciable extent, as in the case of dimethyl sulfate

In order to allow for this side reaction the mixture, from which a portion of the acet-*m*-phenetidine has crystallized, is again subjected to similar treatment with one-half the initial quantities of alkali and diethyl sulfate. It is then allowed to stand overnight after adding 100 cc. of concentrated aqueous ammonia to assist in the decomposition of any remaining excess of diethyl sulfate, the ammonia being alkylated just as any other amine.

Saponification.

The crude acetyl product is filtered off, washed with a little ice-cold water, sucked dry, and boiled one-half hour with 150 cc. of 1 : 1 hydrochloric acid, the acetyl-

[4] Heidelberger and Jacobs, *J. Am. Chem. Soc.* **41**, 1452 (1919).

amino group thus splitting into acetic acid and the amine hydrochloride, which separates on cooling. Enough water is added to dissolve this salt and the solution is then made strongly alkaline with sodium hydroxide and the liberated base is shaken out with ether. The ethereal layer is dried over sodium sulfate or crushed sodium hydroxide, concentrated, and the residue distilled *in vacuo*. 15 g. of *m*-phenetidine should be obtained, boiling at 142-45° under 20 mm. pressure. According to Reverdin and Lokietek[5] a somewhat better yield is obtained using ethyl bromide as the alkylating agent.

D. Allyl Phenyl Ether and its Molecular Rearrangements.

1. Allyl Bromide, $CH_2 : CHCH_2Br$.

The method is essentially that of Merling and Jacobi.[6] 140 g of allyl alcohol, $CH_2 . CHCH_2OH$, are cooled in ice-water. A stream of hydrobromic acid, which may be conveniently generated by dropping bromine on naphthalene and passing the vapors through several bottles containing naphthalene, is then passed in until the liquid is saturated, after which it is boiled under a reflux condenser for one hour, the reaction being:

$$CH_2 : CHCH_2OH + HBr \longrightarrow CH_2 : CHCH_2Br + H_2O.$$

Under the conditions given very little addition of hydrobromic acid takes place at the double bond, although this would undoubtedly occur on long standing of the saturated solution in the cold.

The mixture is then poured into water and the crude bromide separated and washed first with normal sodium

[5] Reverdin and Lokietek, *Bull soc chim* [4] 17, 407 (1915).
[6] Merling and Jacobi, *Ann.* 278, 11 (1894)

hydroxide solution and then with water. After drying over calcium chloride and distilling, the yield should be 85 per cent of the theory, boiling at 70–1°.

2. **Allyl Phenyl Ether**, $C_6H_5OCH_2CH.CH_2$.[7]

94 g of phenol, 121 g. of allyl bromide, 140 g. of dry potassium carbonate, and 150 g of acetone are boiled for eight hours on the water bath under a reflux condenser In this case potassium carbonate is used to combine with the acid liberated in the alkylation, and the reaction is carried on in a non-aqueous solvent Potassium bromide soon separates and the mixture thickens to a paste After cooling, water is added and then ether to take up the allyl phenyl ether. The ethereal layer is shaken out twice with 10 per cent sodium hydroxide solution to remove unchanged phenol. washed with a little water, and then dried over potassium carbonate and distilled *in vacuo* The yield should be 115–30 g., boiling at 85° under a pressure of 19 mm. The purification must be carried out under diminished pressure, as will be seen from the next experiment.

3. ***o*-Allylphenol (*o*-Chavicol).**

$CH_2CH:CH_2$.[8]

DropBooks

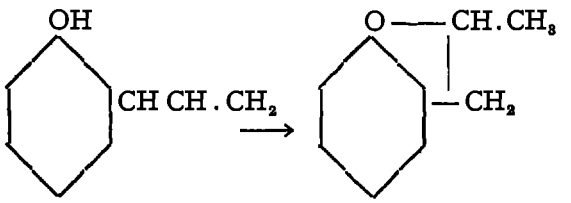
Allyl phenyl ether is boiled under an air condenser until the temperature no longer rises, a process requiring four to six hours. From an initial temperature of about 190° the thermometer finally rises to about 220°. The gradual increase in boiling point is due to a re-

[7] Claisen and Eisleb, *Ann* 401, 21 (1913); 418, 78 (1918).
[8] Claisen, *loc. cit*; Jacobs and Heidelberger, *J. Am Chem. Soc.* 39, 2202 (1917).

markable rearrangement, somewhat analogous to that taking place when the alkyl anilines (in the form of salts) are heated to high temperatures, and consisting in the wandering of the allyl group to the *o*-position in the nucleus. While this reaction takes place comparatively readily in the case of the alkyl aniline salts, the alkyl phenol ethers exhibit no such phenomenon. Apparently the allyl group is the only one possessing such lability in the phenol series.

Purification.

After the boiling has been completed the product is cooled, dissolved in 20 per cent aqueous sodium hydroxide, and the solution shaken with petroleum ether to remove traces of unchanged allyl phenyl ether and small amounts of methylcoumarane, formed according to the following scheme:

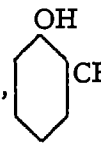


The *o*-allylphenol is then liberated from the alkaline solution by acidification with sulfuric acid, and is taken up in ether, dried over sodium sulfate, and concentrated. Distillation *in vacuo* yields an oil with a guaiacol-like odor, boiling at 109–10° under a pressure of 22 mm and solidifying in a freezing mixture to a mass of crystals which melt at —6°. The yield is almost quantitative

An interesting property of *o*-allylphenol is that, by repetition of the etherification and heating, it can be

converted successively into *o*, *o'*-diallylphenol, and *o*, *o'*, *p*-triallylphenol.

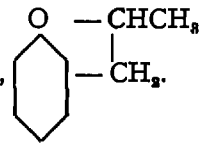
4. ***o*-Propenylphenol (*o*-Anol),**



o-Allylphenol is dissolved in three parts of the strongest possible methyl alcoholic potassium hydroxide and the solution boiled until one part of the methyl alcohol has boiled off, after which a reflux condenser is attached and the mixture boiled in an oil bath for two hours. A similar shifting of the double bond under the influence of alkali is observed in many other cases, and reactions of this type must be carefully avoided in determining the constitution of an unknown substance if the position of any unsaturated linking present is to be fixed with certainty.

The mixture is diluted with water, acidified, and the phenol isolated as in the preceding case. It boils at 230–1° under atmospheric pressure, while according to Pauly and Buttlar[9] it boils at 112–3° under a pressure of 12 mm., solidifying when chilled and rubbed. It may then be recrystallized from ligroin, when it melts at 34·5°.

5. **α-Methylcoumarane,**



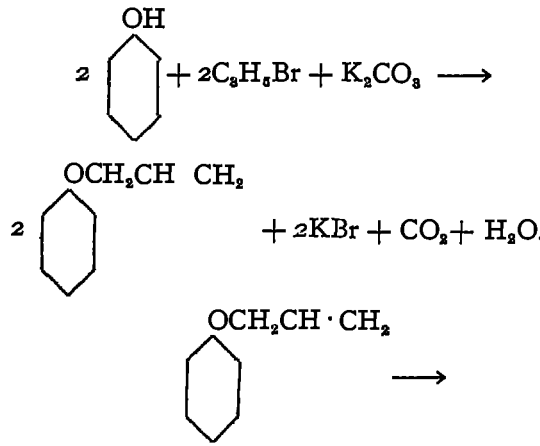
The formation of traces of this substance from *o*-allylphenol was encountered in the preparation of the

[9] Pauly and Buttlar, *Ann.* 383, 280 (1911).

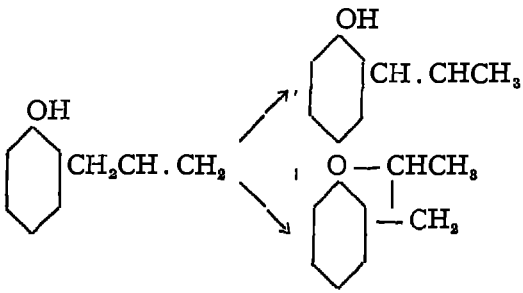
latter, and this remarkable intramolecular rearrangement may be made the chief reaction by using a catalyst, such as pyridine hydrochloride. This salt may readily be obtained by passing dry hydrochloric acid gas through a wide tube into a chilled solution of pyridine in several volumes of benzene.

20 g of *o*-allylphenol are boiled with 2 g. of dry pyridine hydrochloride until the boiling point sinks to a *minimum*[10] The solution is diluted with ether and washed with dilute acid to remove the pyridine salt, with dilute sodium hydroxide to remove any unchanged allylphenol, and then with water. After the ethereal layer is dried over sodium sulfate and concentrated the residue is distilled, the α-methylcoumarane boiling at 197–8°.

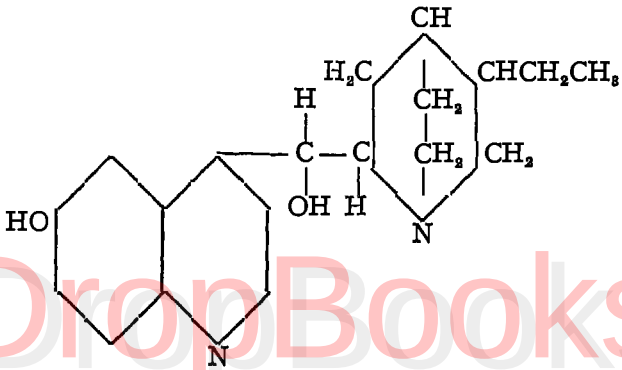
The following scheme will serve to summarize the group of reactions occurring between phenol and allyl bromide



[10] Cf. Ger. pat. 279,864



E. Dihydrocupreine,[11, 12].



DropBooks

[11] A summary of the classic researches leading to the determination of the constitution of the cinchona alkaloids may be found in Schmidt and Grafe's book on "Alkaloide" in Abderhalden's collection "Handbuch der biologischen Arbeitsmethoden" (Part I, Section IX), or better, the original articles of Koenigs, *Ann.* 347, 143 (1906); and Rabe, *Ann.* 365, 353 (1909), 373, 85 (1910). These and Rabe's later articles are only one of the many fascinating and inspiring records afforded by organic chemistry of year-long patient and brilliant investigation, overcoming obstacles one by one, revising ideas originally thought correct, and finally arriving at the true solution.

[12] Heidelberger and Jacobs, *J. Am. Chem. Soc.* 41, 821 (1919).

Demethylation.

50 g of dihydroquinine (p. 50) are boiled with 200 cc. of aqueous hydrobromic acid (d. 1.49), allowing the water boiling off to escape until the temperature has again reached that of the constant-boiling hydrate, when an air-condenser is attached and the boiling continued for four hours The methyl bromide liberated may be collected if desired by leading tubes from the air-condenser into a test tube immersed in a freezing mixture.

Phenolic ethers, of which dihydroquinine is an example, may be dealkylated by heating with acids Hydrochloric acid is often used, but the reaction rarely takes place at the boiling point of the constant-boiling hydrate and is therefore generally carried out in sealed tubes The pressure developed by the methyl chloride liberated often results in the breakage of tubes, but can be avoided to some extent by frequently opening and resealing the tubes Concentrated hydriodic acid is also a useful dealkylating agent, but is relatively expensive, and hydrobromic acid may therefore be used to advantage in many cases especially when large amounts of material are in question. While a solution of the acid in glacial acetic acid is often used, the constant-boiling hydrate (d 1 49) which boils at 126° at 760 mm. pressure, and contains 47.8 per cent of hydrobromic acid, has been found very satisfactory for cases such as the present one.

Filtration of the Dihydrobromide.

The dark brown solution is cooled and allowed to stand overnight in the ice box The heavy, crystalline dihydrocupreine dihydrobromide which separates is filtered off on cloth on a Buchner funnel and washed with a little of the hydrobromic acid. The filtrate is then distilled to about one-third of its original volume, which, besides concentrating the mother liquors, effects

the conversion of any unchanged dihydroquinine present. The crop of crystals collected after cooling the concentrated liquid is filtered off as above and added to the first fraction, while the filtrate may be added to the hydrobromic acid used for another preparation of the same substance, in case this is undertaken.

Conversion to Base.

The combined fractions of the dihydrobromide are dissolved in about 2 liters of warm water, cooled, and cautiously treated with 10 per cent sodium hydroxide solution, with vigorous stirring, until the localized precipitate first formed begins to dissolve slowly. Addition of sodium hydroxide is then continued rapidly with vigorous stirring until the copious precipitate of the base just redissolves in the excess of alkali, solution taking place, of course, by virtue of the phenolic group exposed by the demethylation. In this way the separation of the dihydrocupreine as a gum may be avoided, as this dissolves in excess alkali only with the greatest difficulty. In case any gummy material is formed in spite of all precautions, it may be removed, dissolved in dilute hydrochloric acid, and the above process repeated, adding the alkaline solution to the main portion. Bone black is next added to collect a trace of gelatinous material, and the solution is filtered through large folded filters, for if the manipulations have been properly carried out the alkalinity of the solution should not be so great as to attack the paper. Since alkaline phenolic solutions are subject to oxidation, this part of the preparation should be carried out as rapidly as possible. The base is precipitated from the clear yellow filtrate by the addition of not too large an excess of saturated ammonium chloride solution. The amorphous base is filtered off on a large Buchner funnel, washed with water, sucked as dry as possible,

and added to 190 cc. of boiling 95 per cent alcohol Most of the material dissolves before crystals begin to separate At this point the solution is rapidly decanted from the undissolved substance, which is then dissolved in the minimum amount of boiling alcohol and added to the rest of the solution 25 cc. of water are added and the solution is allowed to cool and stand over night in the ice box, when 23 g. of quite pure dihydrocupreine are obtained On concentration of the mother liquors and washings to about one-half volume and addition of about one-quarter volume of warm water, a further crop of 5 g. of slightly less pure alkaloid is obtained, while about 2 g additional may be recovered by shaking out the ammoniacal filtrate from the crude amorphous base with chloroform

The main fraction, recrystallized from 85 per cent alcohol, yields the pure alkaloid as compact aggregates of thick, minute plates which swell and evolve gas at 185–90°, with preliminary softening, forming a glassy mass which adheres to the walls of the capillary tube and only liquefies completely at 230°, with simultaneous darkening. $[\alpha]_D^{23}$ in absolute alcohol is $-148.7°$, $c = 1\ 13$

The interest attached to this alkaloid lies in the fact that its ethyl ether, the so-called "optochin," is a remarkable and specific bactericide for the pneumococcus, while its homologous ethers are highly bactericidal for other micro-organisms [18]

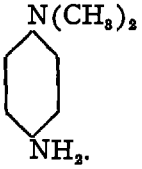
[18] Morgenroth and Levy, *Berl klin. Wochschr.* **48**, 1560 (1911), and later articles

V. REDUCTION.

(*See also p. 93*)

A. Reduction with Stannous Chloride.

***p*-Aminodimethylaniline,**

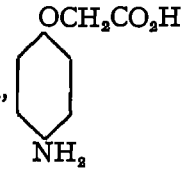


This technically important intermediate may be prepared as follows [1] 50 g. of *p*-nitrosodimethylaniline or a corresponding amount of its hydrochloride, prepared according to the directions in any elementary laboratory manual, are added in small amounts to a warm solution of 225 g. of stannous chloride in 450 cc of concentrated hydrochloric acid, cooling occasionally to prevent the mixture from getting hot during the addition of the amine The reduction is completed by warming for one-half hour on the water bath, after which the mixture is cooled Part of the double tin salt of the aminodimethylaniline is deposited at this point, and the precipitation is completed by saturating the mixture at 0° with hydrochloric acid gas. The salt is then sucked off through a cloth filter and dissolved in water. As the base is readily oxidized by the air

[1] Jacobs and Heidelberger, *J. Biol. Chem.* 21, 113 (1915).

the acid solution is covered by a layer of ether, ice is added, and the compound only then liberated by making very strongly alkaline with 50 per cent sodium hydroxide, being sure that enough ice is present to keep the mixture cold. After shaking cautiously the ether is removed and the alkaline solution again shaken out several times with ether. The combined ethereal extracts are dried over sodium sulfate and sodium hydroxide and concentrated, and the residue is distilled *in vacuo*. The yield of aminodimethylaniline should be 36 g., boiling at 146-8° under a pressure of 24 mm. and solidifying in the receiver to a crystalline mass which melts at 38-41°. Owing to its sensitiveness to the oxygen of the air the base is best preserved in vacuum ampoules (which may be made of thick-walled test tubes), or in glass-stoppered bottles.

B. Reduction with Ferrous Sulfate and Ammonia.

p-Aminophenoxyacetic Acid, 

[2]

20 g. of *p*-nitrophenoxyacetic acid (p. 33) are dissolved in a slight excess of dilute ammonia and the solution is poured in a thin stream into a vigorously rotated, boiling solution of 7 molecular equivalents (one in excess) of ferrous sulfate ($FeSO_4.7H_2O$) in 2 to 2.5 parts of water. Small portions of concentrated aqueous ammonia are then immediately added, rotating the solution vigorously after each addition and continu-

[2] Jacobs and Heidelberger, *J. Am. Chem. Soc.* 39, 1436 (1917).

ing the addition of ammonia until the solution remains definitely alkaline to litmus after shaking and boiling. The mixture is boiled 5 minutes, making certain that the reaction remains alkaline, and is filtered hot through a large Buchner funnel which, with the suction flask, has previously been warmed to prevent cracking. The precipitate of iron hydroxides is washed with a little hot dilute ammonia solution and the filtrate concentrated *in vacuo* until the ammonium salt filtrate of the amino acid begins to separate owing to the salting-out action of the ammonium sulfate present. The mixture is then heated until the salt dissolves and treated with an excess of acetic acid, whereupon 15.7 g. of *p*-aminophenoxyacetic acid precipitate almost at once. The acid crystallizes with one molecule of water of crystallization. Contrary to the statements of others[8] both the air-dry and anhydrous acids melt with gas evolution and resolidification at about 220°, leaving a residue which does not melt below 285°.

The reduction with ferrous sulfate and ammonia takes place according to the equation:

$$O_2NC_6H_4OCH_2CO_2NH_4 + 6FeSO_4 + 12NH_4OH + 6H_2O \longrightarrow H_2NC_6H_4OCH_2CO_2NH_4 + 6(NH_4)_2SO_4 + 6Fe(OH)_3 + 2H_2O.$$

The method is of great value in dealing with substances which are sensitive to strong acids or bases, such as *o*-nitro- and amino-benzaldehydes, nitro- and amino-benzamides and benzoylureas, and is also of service in the preparation of other amino acids.

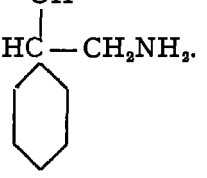
In the reduction of *o*-nitrophenylarsonic acid with the aid of ferrous sulfate, ammonia is not a strong enough base to decompose the iron salt of the amino arsonic acid, and sodium hydroxide must be used.

[8] Howard, *Ber.* 30, 547 (1897); Kym, *J. prakt. Chem.* [2] 55, 113 (1897).

After preparing the *o*-nitrophenylarsonic acid (p 29) this modification may be tried instead of, or in addition to, the above preparation. In the case of arsonic acids the method is of particular utility in that it results only in the reduction of the nitro group, while stronger reducing agents reduce the arsonic acid group as well.

C. Reduction with Sodium Amalgam.

***dl*-Phenylethanolamine, 1-phenyl-1-hydroxy-2-aminoethane,**



The benzaldehyde cyanohydrin required for this preparation is very conveniently made according to Ger pat 85,230 as follows Benzaldehyde is thoroughly shaken, best in a shaking machine for one-half hour, with an excess of concentrated sodium bisulfite solution The crystalline bisulfite addition product, $C_6H_5CH<^{OH}_{OSO_2Na}$, is filtered off, washed with alcohol, and stirred to a thin paste with water. A concentrated solution of 1 1 molecular equivalents of potassium cyanide is then added all at once, with vigorous stirring The bisulfite compound quickly dissolves, and the oily cyanohydrin, $C_6H_5CH<^{OH}_{CN}$, precipitates in al-

most quantitative yield. It is separated from the solution as quickly as possible and used directly for the reduction.

35 g. of the cyanohydrin are dissolved in 550 cc. of 50 per cent alcohol, chilled in a freezing mixture, and reduced with continual chilling and mechanical stirring, with 1400 g. of 4 per cent sodium amalgam, on to all of which the solution should be poured.[4] During the reduction, which requires several hours and proceeds according to the equation:

$$C_6H_5CH\begin{matrix}OH\\CN\end{matrix} + 4Na + 4H_2O \longrightarrow C_6H_5CH\begin{matrix}OH\\CH_2NH_2\end{matrix} + 4NaOH,$$

the reaction is kept just acid to litmus by slowly dripping in first 50 per cent acetic acid, then the glacial acid, regulating the speed of addition by careful tests with litmus paper, as the reaction should neither be allowed to remain alkaline nor become strongly acid. When all of the sodium has been used up the solution is poured from the mercury and concentrated to about one-half volume. The solid mass of salts obtained on cooling is ground up in a mortar, thinned with a little dilute hydrochloric acid, filtered off, and washed with more of the dilute acid The filtrate is shaken out with ether to remove non-basic impurities and is then covered with a layer of ether, made very strongly alkaline with sodium hydroxide, shaken out, and the extraction with ether repeated several times. The ethereal solution is dried over crushed sodium hydroxide and concentrated to small bulk.

The resulting oily base does not crystallize, but

[4] Cf. Ger. pat 183,634.

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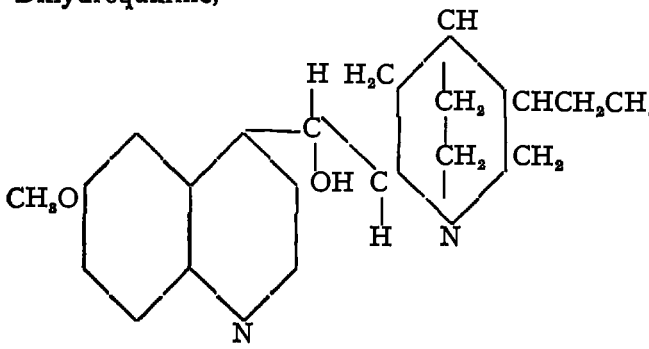
forms a characteristic N-benzoyl derivative It is dissolved in a mixture of 5 parts each (calculating cc per g of base) of glacial acetic acid and saturated sodium acetate solution and shaken in the cold with 1 1 molecular equivalents of benzoyl chloride.[5] The benzoyl derivative separates as an oil which crystallizes after being rubbed Recrystallized from the minimum amount of alcohol it forms nacreous scales which melt at 148.5–9.0°.[6]

D. Reduction with Palladium Black.

Dihydroquinine,



One of the most important methods for the identification of unsaturated linkages, and a method which has had important consequences in the study of ring systems in general and alkaloids in particular, is the Paal-Skita method of reduction with colloidal metals, in which finely divided palladium or platinum acts as

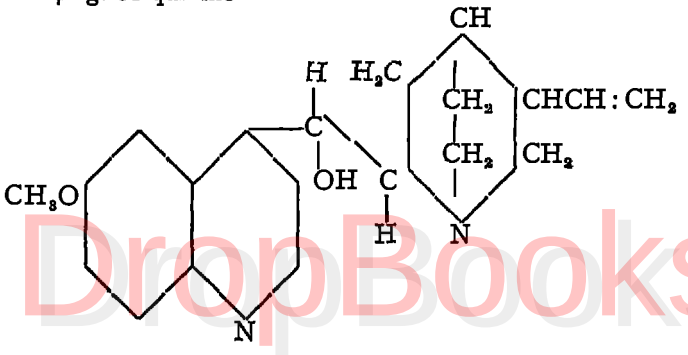
[5] For this general method of acylation see Jacobs and Heidelberger, *J. Am. Chem. Soc* 39, 1439 (1917)

[6] Kolshorn, *Ber.* 37, 2483 (1904), and Rosenmund, *Ber.* 46, 1046 (1913), give 147°.

carrier for the hydrogen either in the presence or absence of a "protective colloid."[7] Many of the modifications of the method have been applied on an enormous scale industrially, as in the hardening of fats with the aid of reduced nickel.

In the cinchona series[8] the method is particularly easy of application and has been used for the production of the dihydro alkaloids, whose biological properties were first extensively studied by Morgenroth and his collaborators.[9]

40 g. of quinine



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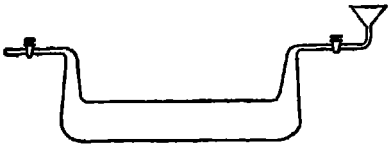
are dissolved in 180 g. of 10 per cent aqueous sulfuric acid, filtered, and treated with 8 to 10 cc. of a 2 per cent solution of palladious chloride[10] prepared by dissolving the salt in a little hot 1:1 hydrochloric acid, concentrating as far as possible on the water bath, and diluting to volume with water. The precipitate which forms in the quinine solution dissolves readily on stir-

[7] For a detailed discussion see Skita's monograph.
[8] See footnote, p. 41.
[9] *Loc. cit.*
[10] Ger. pat 252,136; Heidelberger and Jacobs, *J. Am. Chem. Soc.* 41, 819 (1919).

ring, and the clear solution is then rinsed into a shaking apparatus (that shown in the cut is a convenient form), the air is displaced by hydrogen, and the whole shaken with free access of hydrogen under pressure of a column of water varying from about 25 to 60 cm. The reaction proceeds slowly until the palladium is



entirely reduced, after which the absorption of hydrogen takes place rapidly until almost the calculated amount has been absorbed, slowing down towards the end. The mixture is poured out of the shaker and either centrifuged or allowed to stand until the palladium has settled, after which the liquid is carefully poured off and the palladium washed either by centrifugation or decantation, after which it may be used for four or five additional reductions The solution and washings are diluted with a large volume of water and rapidly made alkaline with 10 per cent aqueous sodium hydroxide, for if the addition of alkali is too slow the sparingly soluble neutral sulfate of dihydroquinine, $(C_{20}H_{26}O_2N_2)_2.H_2SO_4$, may crystallize out and contaminate the base The amorphous precipitate is filtered off on a large Buchner funnel, washed well with water, pressed out and sucked as dry as possible, and then spread out to dry in the air. It is recrystallized by adding to hot dry acetone or toluene, and when dried melts at 171–2° as recorded in the literature for the natural alkaloid which occurs in small amounts associated with quinine.

While quinine in dilute sulfuric acid immediately reduces a one per cent solution of potassium permanganate, the permanganate color persists for some moments in a similar solution of pure dihydroquinine, and this test should be performed as a control of the purity of the product obtained.

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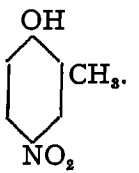
VI. OXIDATION.

A. Oxidation with Potassium Ferricyanide.

p-Nitro-*o*-cresol, 

In the direct nitration of *o*-cresol a mixture of the *p*- and *o*- derivatives is formed, from which relatively little of the *para*- isomer can be isolated.[1] Better methods for the preparation of the *para*-compound are by diazotization of 5-nitro-*o*-toluidine and replacement of the diazo group by hydroxyl, or by simply boiling the nitrotoluidine with strong aqueous alkali.[2]

Another very convenient method which gives a homogeneous product in good yield involves the oxidation of *p*-nitroso-*o*-cresol to the *p*-nitro derivative, as given by Borsche and Berkhout,[3] except that almost quantitative yields are obtained in the cold instead of on boiling as recommended by these authors

15 g. of *p*-nitroso-*o*-cresol (p 16) are dissolved in a solution of 150 g. of sodium hydroxide and 150 g. of potassium ferricyanide in 3 liters of water, and allowed

[1] Hirsch, *Ber* 18, 1512 (1885)
[2] Neville and Winther, *Ber* 15, 2978 (1882)
[3] Borsche and Berkhout, *Ann* 330, 95 (1904).

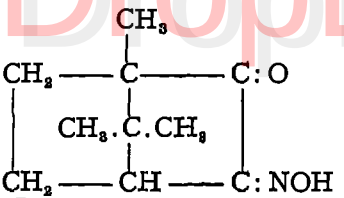
to stand at room temperature for two days. The oxidation may be considered to take place as follows:

$$RNO + 2K_3Fe(CN)_6 + 2NaOH \longrightarrow RNO_2 + 2K_3NaFe(CN)_6 + H_2O,$$

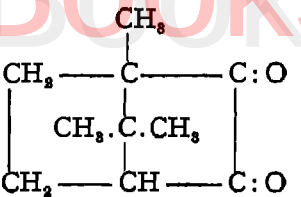
the ferricyanide being reduced to ferrocyanide. The large excess of ferricyanide is necessary in order to insure the completion of the reaction.

The solution is finally acidified with sulfuric acid and extracted several times with ether. The ethereal layer is in turn shaken out with 5 per cent sodium hydroxide solution and this warmed on the water bath to drive out the dissolved ether. On cooling and acidifying with sulfuric acid the nitrocresol separates as faintly yellow needles melting at 93–5° after drying *in vacuo* over sulfuric acid. The yield should be 15 g.

B. Oxidation with Nitrites: Isonitrosocamphor and Camphorquinone.[4]



Isonitrosocamphor

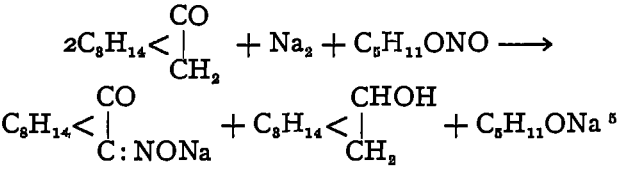


Camphorquinone

56 g. of camphor (natural) are dissolved in 250 cc. of dry ether in a large flask and 7.6 g. of sodium wire added. The mixture is cooled with ice water, after which 39 g. of isoamyl nitrite are added in small portions with shaking and continued cooling, the addition

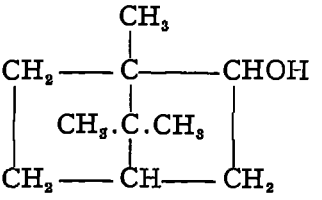
[4] Cf. Claisen and Manasse, *Ann.* 274, 73 (1893).

of the nitrite taking 10–15 minutes It should also be remembered that isoamyl nitrite is quite volatile and extremely toxic The reaction may be considered to take place as follows



Purification.

After several hours ice water is added (if there is no sodium left), giving a reddish solution of the sodium salt of the isonitroso compound Unchanged camphor and borneol,



formed by reduction are removed by shaking out cautiously several times with benzene, and the aqueous layer is filtered through a wet paper to remove traces of solvent. Dilute acetic acid is then added until no further precipitation occurs The yield of crude product, which is, of course, a mixture of isomers, should be about 22 g.

[5] Cf Baubigny, *Ann chim phys.* [4] 19, 221 (1870), Beckmann, *Ber* 22, 912 (1889), for a discussion of the action of sodium on camphor,

Isomerization.

Owing to the presence of a low-melting isomer, Claisen and Manasse had much difficulty recrystallizing the compound to constant melting point, but Forster [6] found that the low-melting isomer is converted into the high-melting form when the dried mixture is heated at 150° for a few minutes. After recrystallizing the melt from boiling ligroin the isonitrosocamphor melts at 152-3°.

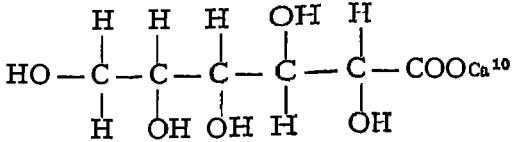
Fission.

12.5 g of isonitrosocamphor are dissolved in 22.5 cc. of glacial acetic acid and treated slowly with 50 g. of finely powdered, hydrated sodium sulfite, while the solution is shaken vigorously and heated over a free flame.[7] When all of the sulfite has been added the mixture is boiled for one hour under a reflux condenser, using a sand bath. According to von Pechmann [8] the sodium bisulfite initially formed reacts with the isonitroso compound as follows:

$$\begin{array}{l} \mathrm{R.CO.C.NOH} + \mathrm{NaHSO_3} \longrightarrow \\ \quad\quad\quad\

$$\begin{array}{l} \mathrm{R.CO.C:NOSO_2Na} + 2\mathrm{H_2O} \longrightarrow \\ \quad\quad\quad\

D. Oxidation with Bromine: Gluconic Acid Calcium Salt,



To a solution of 200 g. of anhydrous glucose in one liter of water add gradually 200 g. of bromine, with frequent shaking. About 50 g. of the bromine may be added at once and then the rest in 25-50 g. portions as the fluid bromine disappears into solution. Kiliani's original method calls for double the amount of bromine, but Ruff [11] found that an equal weight was sufficient. It is well, however, to control the progress of the oxidation by the gradual diminution in reducing power (Fehling's solution) of the solution, and to use a little more bromine if the reducing powder has not fallen to a minimum after an equal weight of bromine has reacted. The oxidation may be considered to take place according to the equation:

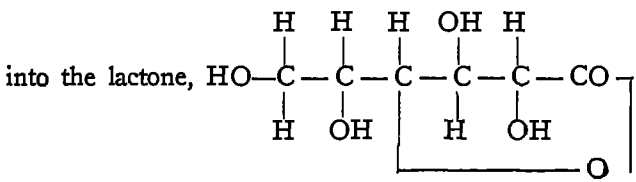
$$RCHO + Br_2 + H_2O \longrightarrow RCOOH + 2HBr.$$

After the reaction is over the excess of bromine is boiled off, stirring to prevent local overheating, and the golden yellow solution is cooled and the volume measured. Bromine ion is then determined in an aliquot portion, after which the calculated amount of lead carbonate, ground to a thin paste with water, is added in small portions, with stirring. An appreciable excess of lead carbonate is to be avoided, as lead gluconate is

[10] Kiliani, *Ber* 17, 1298 (1884).
[11] Ruff. *Ber.* 32, 2273 (1899).

then formed and this prevents the lead bromide from crystallizing out, presumably owing to the formation of a double salt. The resulting mixture is concentrated, best *in vacuo*, to 500 cc., chilled, and allowed to stand in the ice box for 24 hours, after which the lead bromide is filtered off and washed with a little ice-cold water. Traces of bromine ion in the filtrate are removed by adding a little freshly precipitated silver oxide or a suspension of silver carbonate, and after filtration hydrogen sulfide is passed in to remove minute amounts of lead and silver ions in solution. Any sulfides formed are filtered off and the filtrate, which now consists of a solution of gluconic acid, is boiled with an excess of calcium carbonate After cooling, and filtering off the excess of carbonate, the filtrate is concentrated *in vacuo* to a thin syrup. The calcium salt of gluconic acid separates on cooling and letting stand, with occasional rubbing, and is filtered off and recrystallized from the minimum amount of boiling water or dilute alcohol. The yield of crude calcium salt should be 140–50 g.

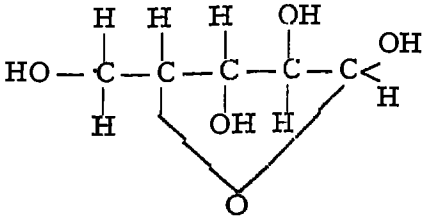
The free gluconic acid is a syrup and passes over into the lactone,



from which crystals have been obtained on long standing[12]

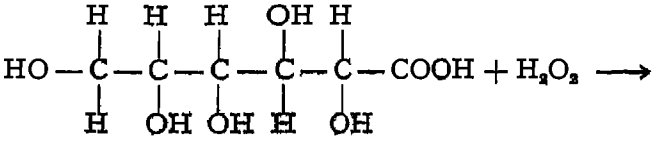
[12] Kiliani, *loc. cit.*, footnote, p 1300

E. Oxidation with Hydrogen Peroxide: d-Arabinose,[18]

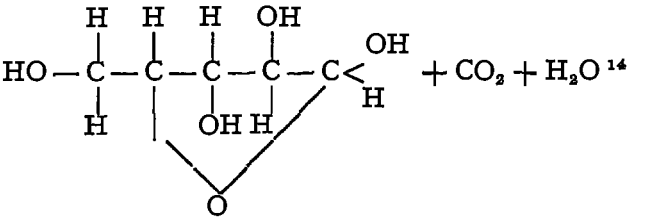


(α- and β-).

125 g. of calcium gluconate are dissolved in 375 cc. of hot water, cooled to 35°, and treated with an amount of hydrogen peroxide solution corresponding to 1.5 atoms of active oxygen, and 25 cc. of a basic ferric acetate solution containing 5 per cent of iron (prepared by adding with vigorous stirring, a solution of 18 g. of ferric sulfate in 500 cc. of water to 42.5 cc of concentrated aqueous ammonia diluted with 150 cc. of water. After allowing to settle, sucking off and washing free from ammonia (Nessler test) with hot water, the precipitate, which should be pressed down on the funnel until it weighs less than 35 g., is added to 13 g. of acetic acid, stirred until dissolved, and diluted to 50 cc). The evolution of carbon dioxide which accompanies the reaction, according to the equation:



[18] Ruff, *Ber.* 32, 553 (1899); 35, 2360 (1902).



is over in about 6 hours, and all the hydrogen peroxide is then used up The calcium acetate and ferric hydroxide are filtered off and the solution is concentrated to a thick syrup *in vacuo* This is kneaded thoroughly with 500 cc. of alcohol until the undissolved calcium salts form a crumbly mass. The solution is poured off and the salts, which occlude appreciable amounts of arabinose, are dissolved in the minimum amount of hot water and again kneaded with a smaller amount of alcohol, repeating the solution and reprecipitation of the salts until a test portion of the alcoholic solution, when concentrated, no longer appreciably reduces Fehling's solution. The alcoholic extracts are combined and concentrated again *in vacuo* to a thick syrup. This is boiled out repeatedly with 90 per cent alcohol, seeding the chilled filtrates with arabinose if necessary in order to start crystallization The yield of fairly pure sugar so obtained should be 18–21 g. An additional 5 g. may be recovered by taking the calcium salts remaining from the alcoholic extraction, which contain unchanged calcium gluconate, and putting them through the entire process again, using one-fourth of the initial amount of hydrogen peroxide and ferric acetate 2 5 g. more may also be isolated from the mother liquors of the crystalline arabinose by concentrating as far as

[14] A ketonic acid is possibly the intermediate stage Ruff, *Ber* 31, 1574 (1898)

possible *in vacuo*, taking up in 95 per cent alcohol, adding one-quarter volume of ether, and filtering off the precipitated calcium salts quickly with the aid of bone-black before the arabinose begins to separate.

The sugar may be freed from traces of ash by recrystallization from one-half its weight of water, with the aid of bone-black. It should then form rhombic prisms which melt at 158.5–9.5° when anhydrous and show $[\alpha]_D^{20} = -105.1°$ on coming to equilibrium ($c = 9.4253$).

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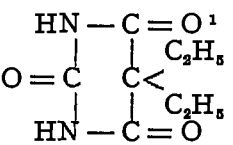
VII. FORMATION OF HETEROCYCLES AND DYES.

A. Diethylbarbituric Acid, "Veronal," "Barbital,"



1. Diethylmalonic Ester, $(C_2H_5)_2C(CO_2C_2H_5)_2$.

The essential requirement in the preparation of diethylmalonic ester and "veronal" is that all reagents must be absolutely anhydrous, otherwise the yields will be vanishingly small. All reagents and solutions must therefore be protected from the moisture of the air after drying.

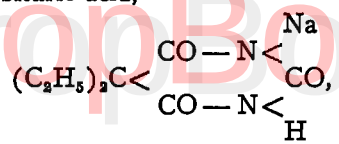
Required are 100 g. of carefully dried and freshly distilled malonic ester, prepared according to the directions in the elementary text books, 28 8 g. of sodium (2 molecular equivalents), 380 cc of absolute alcohol (dried by adding to ordinary absolute alcohol enough sodium to combine with 1 per cent of water and distilling), and 195 g of carefully dried and freshly distilled ethyl iodide (2 molecular equivalents). One-half of the sodium is dissolved in one-half of the alcohol under a reflux condenser surmounted by a calcium chloride tube, the malonic ester added *with stirring*, and then

[1] The writer is indebted to Dr F A. Taylor of the Rockefeller Institute for Medical Research for placing at his disposal the results of working over the method of Fischer and Dilthey, *Ann.* 335, 338 (1904).

slowly through a dropping funnel 1 equivalent of the ethyl iodide. The mixture is finally boiled, and when neutral to moistened red litmus the second half of the sodium is dissolved in the remainder of the alcohol, the neutral reaction mixture added, and the balance of the ethyl iodide slowly dripped in, after which the mixture is boiled over night, when it should again be neutral. As much alcohol as possible is distilled off, ether and water are added, the two layers separated, and the ethereal solution is dried over the sodium sulfate. 112 g. of ester boiling at 220-2° should be obtained, or 83 per cent of the theory, and on redistillation, 102.5 g. boiling at 221–2°.

2. **Diethylbarbituric acid.**

16 g. of sodium are dissolved in 300 g. of absolute alcohol (dried as in *1*), the solution cooled to room temperature, and 50 g of diethylmalonic ester added. 20 g. of pulverized and carefully dried urea are then dissolved in the mixture by gentle warming and the whole is heated in an autoclave at 108° for 5 hours (internal temperature) The precipitated sodium salt of diethylbarbituric acid,

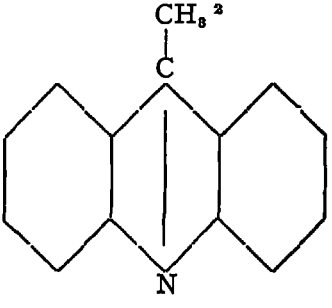


is filtered off, washed with alcohol, dissolved in water, and converted into the free acid by adding concentrated hydrochloric acid. After recrystallization from water the yield is 27.5 g. melting at 183–5°, while Fischer and Dilthey give 191° (corr.) for the pure product.

The acid so obtained is not quite pure, and is recrystallized from 95 per cent alcohol. About one-half of

the quantity recrystallized is obtained in the first crop, and the purity of this should be controlled by melting-point determinations and analyses for carbon, hydrogen, and nitrogen (Kjeldahl). The fractions obtained by concentrating the mother liquors do not give such satisfactory analyses

B. 9-Methylacridine,



50 g. of diphenylamine, 30 cc. of glacial acetic acid, and 85 g of anhydrous zinc chloride are slowly heated to 100° in an oil bath and kept at that temperature for about one hour, in order to insure maximum conversion into N-acetyldiphenylamine The melt is then gradually heated to 220° and kept at that temperature for 14 hours, with occasional stirring This results in dehydration of the acetyl derivative with formation of the acridine ring, but the reaction does not proceed quantitatively in the desired sense, and considerable amounts of tarry by-products are formed When the melt has cooled to about 100° it is dissolved in 50 per cent sulphuric acid, keeping the mixture hot until dissolved, and poured into a rapidly rotated flask containing water. The fluorescent greenish-yellow solution is poured into

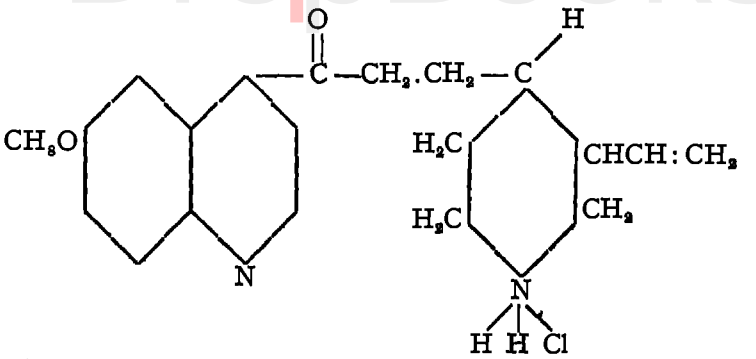
[1] Cf Bernthsen, *Ann.* **224**, 34 (1884).

a large vessel and the resinous residue extracted 4–6 times with hot, very dilute sulphuric acid, adding the solution to the original aqueous extract. The cold acid solution is finally filtered with the aid of bone-black and the 9-methylacridine precipitated by adding ammonia. It is apt to separate amorphous at first, but crystallizes on standing, and is then filtered off, washed with water, and recrystallized from 85 per cent alcohol. It forms greenish brown needles which melt at 114°, and the yield should be about 25–30 g Further amounts may be obtained by diluting the alcoholic mother liquors and recrystallizing the impure product which separates.

In order to obtain the base in a state of absolute purity Koenigs [8] dissolves 10 g. in 50 cc. of alcohol and adds a solution of 10 g of tartaric acid in 100 cc. of alcohol. The tartrate which separates is washed with alcohol, dissolved in water, and converted into the base with ammonia. The acridine should then form greenish yellow needles which melt at 117–8°.

DropBooks

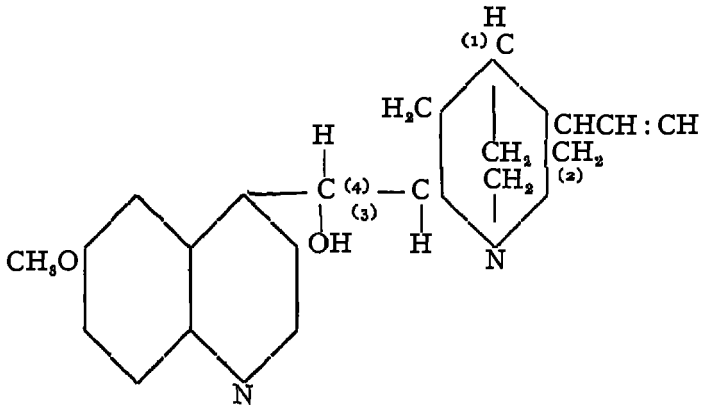
C. Quinicine (Quinotoxine) Hydrochloride,



[8] Koenigs, *Ber.* 32, 3607 (1899).

50 g. of quinine or quinidine (calculated to the anhydrous basis) are dissolved in 100 cc. of 50 per cent acetic acid and 600 cc of water and boiled for 32 hours under a reflux condenser.

Rabe [4] has found that quinine and quinidine



are stereoisomers differing in the spatial relationships about the asymmetric carbon atom (3) and identical as far as asymmetric carbon atoms (1) and (2) are concerned.[5] Therefore, in the remarkable intramolecular change into quinicine effected as above by boiling with weak acids, or by heating the acid sulfate of the base, it would be expected that both quinine and quinidine would yield the same quinicine, as the asymmetry about carbon atoms (3) and (4) disappears. This is actually found to be the case; and it is accordingly a matter of indifference whether quinine or quinidine be used as starting material.

[4] Rabe, *Ann.* 373, 91 (1910).

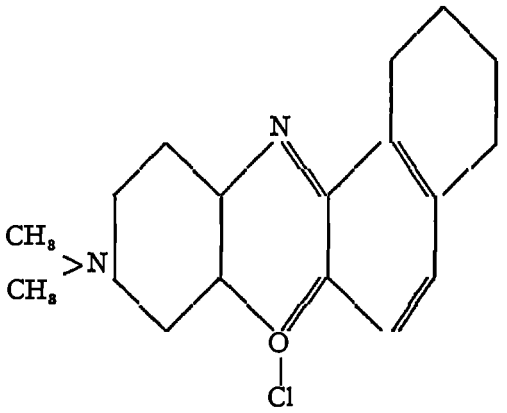
[5] For a possible elucidation of the asymmetry at (4), see King and Palmer, *J. Chem. Soc.* 121, 2577 (1922).

The solution, which is now colored a deep brownish orange, is chilled, made alkaline with sodium hydroxide, and the base extracted with ether. As quinicine itself has never been obtained crystalline it is isolated as the hydrochloride.[6] The ethereal solution is accordingly carefully dried over crushed sodium hydroxide, concentrated, and the viscous residue taken up in about 3 volumes of absolute alcohol. It is then neutralized, with cooling, by carefully adding absolute alcohol saturated at 0° with dry hydrochloric acid gas, until a test drop proves neutral to wet litmus paper. It is a good plan to keep about one-tenth of the solution separate, and if the endpoint is accidentally slightly overrun, an excess of the base can be restored by adding the portion withdrawn. The neutral solution should be protected from the moisture of the air and allowed to stand in the ice-box with occasional rubbing. In case the salt does not crystallize under these conditions crystals for seeding may be obtained by adding dry ether to a little of the solution until just turbid, and letting stand in a well-stoppered test tube.

The yield should be 37 g. Recrystallized from absolute alcohol the hydrochloride forms aggregates of minute leaflets which melt with decomposition at 180–2° and give $[\alpha]_D^{20} = +13.7°$ in water, $c = 1.861$, while anhydrous quinine hydrochloride has a specific rotation of $-149.8°$ and anhydrous quinidine hydrochloride one of $+200.8°$ in water.

[6] Heidelberger and Jacobs, *J. Am. Chem. Soc.* 41, 832 (1919).

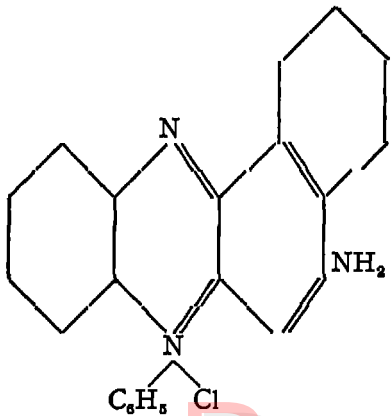
D. Meldola's Blue,



This representative of the oxazine dyes is prepared as follows: 15 g. of β-naphthol are dissolved on the water bath under a reflux condenser in 50 g. of 95 per cent alcohol. To the boiling solution is gradually added a solution of 15 g. of *p*-nitroso-dimethylaniline hydrochloride in 100 g. of hot 50 per cent alcohol, care being taken that boiling does not cease. As soon as the mixture becomes a pure violet in color (about 20 minutes) it is removed from the bath and allowed to stand over night at room temperature Longer heating does not improve the yield. The oxazonium chloride separates as black needles with a brassy reflex, and is sucked off and washed with a little alcohol. To the 7 g of impure dye so obtained 3 g. may be added by cautious addition of an equal volume of ether to the filtrate. The dye is analytically pure only after two recrystallizations from alcohol, and then forms narrow, black platelets with a greenish metallic luster. It then sinters slightly, but does not melt,

when heated to 265°. The aqueous solution is violet in color.

E. Rosinduline,

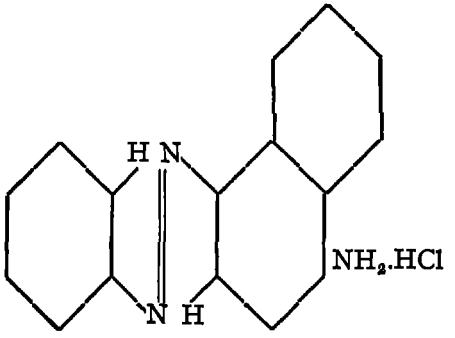


This representative of the large group of phenazine dyes is prepared essentially according to Fischer and Hepp [1] as follows:

28 g. of aniline are diazotized in the cold with 75 cc. of water, 75 cc. of concentrated hydrochloric acid, and 21 g of sodium nitrite in a little water, and the solution is added slowly, with stirring, to a lukewarm solution of 43 g of α-naphthylamine in 500 cc. of 90 per cent alcohol. In order to insure complete conversion of the resulting pasty mass of the dye into the hydrochloride 200 cc. of 1:1 hydrochloric acid are added and the stirring continued for one-half hour.

One part of the dried phenylazo-α-naphthylamine hydrochloride, 2 parts of aniline, and 4 of alcohol are heated for 6–8 hours at 160–70° in sealed tubes, the condensation probably taking place as follows:

[1] Fischer and Hepp, *Ann.* 256, 236 (1890).

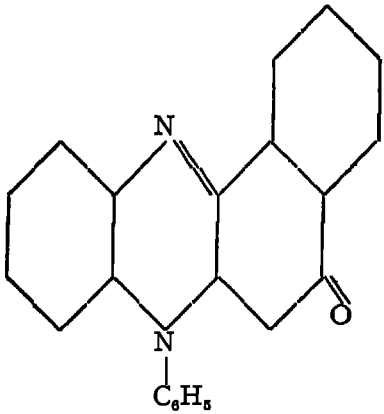


$$\longrightarrow \text{Rosinduline} + NH_3 + 2H,$$

the hydrogen not appearing as such, but instead reducing a portion of the azo dye.

The contents of the tubes are distilled with steam to remove aniline and alcohol, and the residue is boiled out repeatedly with water. The intensely colored solution so obtained is allowed to stand for several days, poured off from the tar which settles, acidified with hydrochloric acid, and the dye precipitated by the careful addition of salt. It forms long red needles with a green reflex, and contains 3 5 molecules of water of crystallization. To be obtained analytically pure it must be repeatedly purified by solution in hot water, and crystallized by carefully adding saturated salt solution to incipient turbidity, and seeding, washing the crystals so obtained with small amounts of ice-cold water.

A by-product, rosindone,



which is also formed in the reaction and is insoluble in water may be isolated from the insoluble tarry residue by extracting with alcohol and recrystallizing the product from a mixture of alcohol and toluene. It forms red hexagonal plates with a slight green reflex, melting at 259°.

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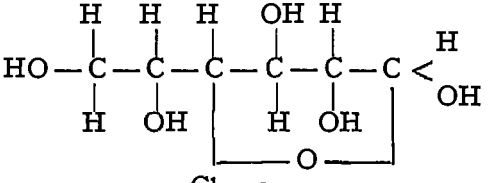
VIII. SUGARS, PROTEINS, AND AMINO ACIDS.

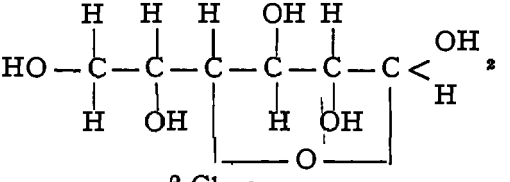
A. Separation of Stereoisomers— β-Glucose from Glucose.[1]

The pyridine required for this preparation must be absolutely anhydrous, and may be obtained by boiling pyridine with an excess of calcium oxide for 5 or 6 hours and distilling immediately before use

12 g. of anhydrous, crystalline glucose, which consists of an equilibrium mixture of the α- and β- forms,



α-Glucose



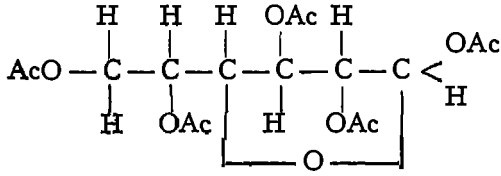
β-Glucose

[1] Cf. Behrend, *Ann* 353, 106 (1907). Thanks are due Dr. G. M Meyer of the Rockefeller Institute for Medical Research for several improvements on the original methods in this and section B.

[2] Cf. Boeseken, *Ber* 46, 2622 (1913); Pictet, *Helvetica Chim. Acta* 3, 649 (1920).

are dissolved in 30 g. of hot, dry pyridine, boiled for 10 minutes under a reflux condenser surmounted by a tube containing calcium oxide, cooled to body temperature, and shaken in a shaking machine in a cool room until crystallization has taken place. After having stood over night the crystalline deposit of β-glucose is filtered off and washed first with a little dry pyridine, then with absolute alcohol, and finally with dry ether. After drying in a desiccator to remove the pyridine of crystallization, the yield should be about 4 g. of sugar melting at 148–50°. When dissolved in water, β-glucose exhibits mutarotation, that is, it slowly reverts to the original equilibrium mixture, and this change may be followed by observing the optical rotation of an aqueous (for example, 4 per cent) solution from time to time until the constant equilibrium value has been attained. For instance, Behrend read the rotation after 8, 18, 28, 38, etc., minutes, and found that equilibrium was attained within 23 hours at a value of $[\alpha]_D = +52.7°$. From the curve plotted from his observations, the value of pure β-glucose by extrapolation to 0 time was found to be $+20.7°$. The student should repeat these observations and check up the values so obtained with those given in Behrend's article.

β-Glucose Penta-acetate.[8]



[8] Cf. Behrend, *loc. cit.*

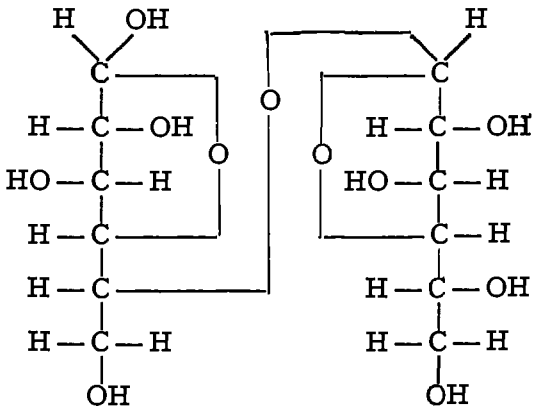
1.8 g. of finely powdered β-glucose are added to an ice-cold mixture of 7 g. of acetic anhydride and 10 g. of dry pyridine The mixture is frequently shaken with occasional cooling until all the sugar is dissolved, after which it is allowed to stand at room temperature for 2 days. It is then poured into 100 g. of a mixture of ice and water and stirred vigorously until the gummy precipitate crystallizes The acetate is sucked off after 2 hours, dried in a desiccator, and recrystallized from 40 cc. of 95 per cent alcohol. The yield should be about 2.9 g., melting at 130-1°. According to Hudson and Dale[4] $[\alpha]_D^{20}$ of a 7 per cent solution in chloroform is + 3.8°.

The corresponding α- compound obtained similarly from α-glucose[5] melts at 111°, and gives a value of + 101.6° for $[\alpha]_D^{20}$ in chloroform.

B. Hydrolysis of a Biose— Galactose from Lactose.

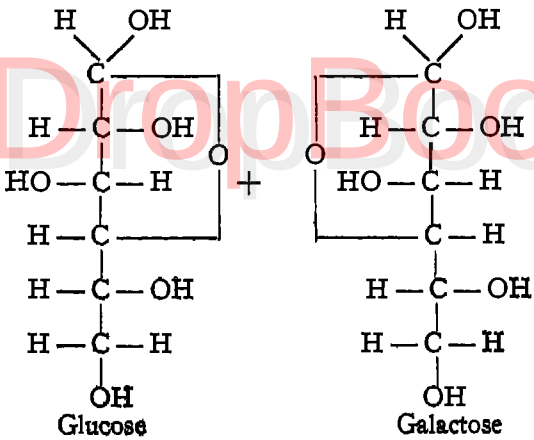
200 g. of milk sugar are dissolved in 500 cc. of warm water. 5.5 cc. of concentrated sulfuric acid are added, and the solution is boiled under a reflux condenser for 2 hours, hydrolysis occurring according to the scheme:

[4] Hudson and Dale, *J Am. Chem Soc.* 37, 1265 (1915).
[5] Behrend and Roth, *Ann* 331, 379 (1904).



Lactose

$+ H_2O \longrightarrow$



Glucose Galactose

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The mixture is boiled a few moments longer with bone black to remove most of the color developed, and filtered hot. The sulfuric acid is then *quantitatively* removed by means of barium hydroxide, of which about 30 g. of the dried base are necessary. The solid compound can be added in the beginning, finishing with a hot, concentrated solution and avoiding an excess of alkali, to which the hexoses are very sensitive. 6 cc. of glacial acetic acid are then added, the mixture is again decolorized with bone black, filtered, and concentrated *in vacuo* to a thick syrup, of about 120 cc. in volume, immersing the flask in a water bath, the temperature of which does not exceed 60-70°. Before the syrup cools, one to one and one-half volumes of glacial acetic acid are added, and the mixture is stirred well, cooled, and rubbed, or better, seeded with a crystal of galactose. After three hours the sugar is sucked off on a Buchner funnel and washed with a little cold acetic acid, then with a very little alcohol, and finally with ether. The yield should be 50 g.

The purity of the galactose obtained should be controlled by a determination of the optical rotatory power. $[\alpha]_D^{20}$ of a 10 per cent solution of the pure sugar at equilibrium in water is $+81.5°$.

C. Preparation of *dl*-Alanine, $CH_3CHNH_2CO_2H$.[6]

While the alanine obtained by hydrolysis of proteins is the *dextro*- isomer, synthetic methods, of course, yield the *dl*- mixture, from which the *d*- isomer may be separated as given below.

50 g. of α-bromopropionic acid (p. 20) are slowly

[6] Acknowledgment is due Dr L. A. Mikeska of the Rockefeller Institute for Medical Research for details of this method and of slight modifications of Fischer's procedure in the following preparations.

stirred into two volumes of concentrated aqueous ammonia cooled by means of a freezing mixture. The solution is then transferred to an autoclave and heated at 100° for three hours, after which it is concentrated to dryness *in vacuo*. The ammonium bromide in the residue is extracted by heating repeatedly with small portions of 95 per cent alcohol and decanting the hot solution, until a test shows that very little more is being removed. The residue of crude alanine is dissolved in the minimum amount of hot water and about five volumes of alcohol are then stirred in, causing the alanine to crystallize quickly. The product is filtered off, dried, and the purity controlled by a nitrogen determination (Kjeldahl) and a test for bromine ion, as well as a determination of amino nitrogen by the Van Slyke or Soerensen method The yield should be about 50 per cent of the theory.

D. Separation of *dl*-Alanine into its Optical Isomers.[7]

Of the methods for the separation of racemic mixtures into their components one of the most useful is the formation of salts with optically active acids or bases. Early attempts to apply this method to the amino acids failed, since these compounds are not only weak acids, and therefore form rather unstable salts with bases, but are also weak bases, and form unstable salts with acids. Fischer overcame this difficulty by converting the amino acids into their benzoyl derivatives, which he found to be stronger acids and so formed stable salts with optically active bases such as the alkaloids strychnine and brucine. Ordinary methods of benzoylation failed in this instance, but good yields were obtained in the presence of sodium bicarbonate.

[7] E. Fischer, *Ber.* 32, 2451 (1899).

1. Benzoylation of Alanine.

To a solution of 45 g. of *dl*-alanine in 450 cc. of water 330 g. of sodium bicarbonate are added, and then 218 g (3 mols) of benzoyl chloride in about ten portions The reaction mixture is shaken vigorously and frequently cooled with ice-water. When all of the chloride has been added the mixture is allowed to stand at room temperature for 4 to 5 hours, with frequent shaking, and is then filtered Any benzoyl chloride in the filtrate is shaken out with ether, after which the aqueous layer is acidified to Congo red with 1.1 hydrochloric acid, causing the separation of a mixture of benzoic acid and benzoylalanine. After letting stand over night in the cold the crystals are collected, dried, and boiled out repeatedly with ligroin to remove the benzoic acid. The yield of crude product should be over 90 g. After recrystallization from water, 70 g. of pure benzoylalanine, melting at 165-6° (corr.), are obtained.

2. *l*-Benzoylalanine.

65 g. of the *dl*-benzoyl compound and 157 g of brucine (hydrate) are dissolved in 240 cc. of hot water After 15 hours at 0° the brucine salt which has separated is filtered off and purified by recrystallizing twice from 100 cc. of water, filtering each crop of crystals only after 12 hours in the ice box. The purified salt forms radiating masses of long, pointed plates, and should be obtained in a yield of about 87 g.

Recovery of Brucine.

80 g. of the brucine salt are converted into *l*-benzoylalanine by dissolving in 240 cc of hot water and stirring in 140 cc. of normal sodium hydroxide solution. The mixture is cooled to 0° in order that the brucine may be as completely removed as possible, and

the alkaloid is filtered off and washed with a little ice-cold water. The filtrate is then acidified with 140 cc. of normal hydrochloric acid solution, concentrated to small volume *in vacuo* at 40-50°, and cooled. 19 g. of *l*-benzoyl derivative should separate as an oil which soon crystallizes. Recrystallized from 5 parts of water it forms glistening plates which melt at 150-1° (corr.), considerably lower than the racemic compound. If the crystallizations have been carefully carried out and the benzoyl compound is free from the *d*-isomer, $[\alpha]_D^{20}$ of a solution of 0.75 g. in 3.9 cc. of normal potassium hydroxide solution and 2.9 cc. of water should be $-37.3°$.

3. *l*-Alanine.

5 g of the benzoyl derivative are hydrolyzed by heating with 25 cc. of 20 per cent aqueous hydrochloric acid in a boiling water bath for 5 hours. The benzoic acid and unchanged benzoylalanine which crystallize on cooling are shaken out with ether, after which 1 g. of benzoylalanine may be rocevered from the ethereal residue as in the original preparation of this substance. The aqueous acid solution containing the hydrolyzed amino acid is evaporated dry, leaving a crystalline residue of *l*-alanine hydrochloride. This is purified by solution in a small amount of warm absolute alcohol, addition of a few drops of alcoholic hydrochloric acid, and gradual addition of ether. The resulting delicate needles are dissolved in 30 parts of water and boiled 15 minutes with an excess of lead oxide prepared by pouring lead acetate solution into warm barium hydroxide solution in excess, filtering, and washing. After the alanine salt–lead oxide mixture has been boiled and a filtered test portion shows only traces of chlorine ion the mixture is cooled and

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filtered. The filtrate is freed from lead salts with hydrogen sulfide, and evaporated to dryness The almost pure *l*-alanine so obtained is dissolved in 5 parts of water, warmed on the water bath, and treated with alcohol until crystallization begins. The amino acid separates as rods or prisms on cooling. When rapidly heated it melts at 297° with vigorous gas evolution. An 8 8 per cent solution in water rotates the plane of polarized light 0 21° to the left.

4. *d*-Alanine.

The mother-liquor from the first crop of the crude brucine salts of *l*-benzoylalanine contains the *d*-compound together with smaller amounts of the *l*-compound which render purification as the brucine salt extremely difficult The following procedure is accordingly adopted: The original mother-liquor is diluted with two volumes of water, made alkaline with 250 cc. of normal sodium hydroxide solution, and cooled. The alkaloid is filtered off and the filtrate again acidified with 250 cc. of normal hydrochloric acid solution. After one day 9 g of *dl*-benzoylalanine separate, which are of no use as far as the preparation of the *d*-compound is concerned. The filtrate is concentrated to small volume *in vacuo*, yielding about 18 g of a crystalline mixture consisting of about 90 per cent of *d*-benzoylalanine and 10 per cent of the *l*-compound.

This is best separated by means of the strychnine salts. 13.3 g. of the crystals and 23 g of strychnine are dissolved in 300 cc. of hot water and allowed to stand over night in the ice box. The strychnine salt of *d*-benzoylalanine separates as thick platelets, but must be recrystallized four times from the same volume of water before the optical rotation reaches a constant value and all traces of the *l*-compound are eliminated. The yield should then be about 20 g.

d-Benzoylalanine.

A solution of the pure strychnine salt in 600 cc. of water is made alkaline with 40 cc. of normal sodium hydroxide solution and chilled. The strychnine is filtered off and the filtrate acidified with 40 cc. of normal acid and concentrated to small bulk *in vacuo*. 5 g. of pure *d*-benzoylalanine separate on cooling. The melting point should be 150-1° (corr.), while $[\alpha]_D^{20}$ of a 10 per cent solution containing one equivalent of potassium hydroxide is $+37.1°$.

d-Alanine.

Hydrolysis of the *d*-benzoyl derivative is accomplished as in the case of the *l*-compound, and the *d*-alanine is isolated in the same way. It is very similar in its properties to the *l*-compound except that the slight rotation of polarized light is in the opposite direction. The synthetic *d*-alanine is identical in every respect with the *d*-alanine isolated from the hydrolytic products of silk and other proteins.

DropBooks

E. Crystalline Egg Albumin.[8]

The initial requirement for the success of this preparation, which may, in any event, require several weeks for completion, is that the eggs used be not more than two or three days old. The albumin originally present in the egg rapidly undergoes a modification which soon

[8] Hopkins, *J. Physiol.*, 25, 306 (1899-1900). Soerensen, *Compt. rend. lab. Carlsberg*, 12 (1917); *Chem. Abstrs.* 12, 2575 (1918).

Acknowledgment is gratefully made to Dr. Lillian E. Baker of the Rockefeller Institute for Medical Research for the use of her notes on this preparation. Hopkins' original method, modified in several details, and made more precise, was found to give crystalline preparations more consistently than Soerensen's modification.

makes it increasingly difficult and finally impossibl to obtain crystals of this sensitive protein. As few a four fresh eggs may be used, but the conditions c crystallization are somewhat more easily realized whe more are taken.

Removal of Globulins.

The whites of the eggs are carefully separated fro all traces of yolk and the volume is measured. A equal amount of saturated ammonium sulfate solutio is slowly added while the mixture is beaten with a egg-beater in order to break up the membranes enclo ing the substance of the egg-white. To ensure com plete precipitation of the globulins the mixture i allowed to stand over night in the cold, and is the filtered through large folded filters, or better, cen trifuged, as the precipitated globulin rapidly clogs th filters. The volume of the clear yellowish filtrate measured and recorded.

Crystallization.

10 per cent acetic acid is next added slowly fro a burette, with vigorous stirring, until a permanen well-defined precipitate fills the liquid in amount sui ficient to make it actually milky in appearance, nc merely opalescent. When this point has been reache 1 cc of 10 per cent acetic acid for each 100 cc. recorde above is slowly stirred in, giving rise to a heav amorphous precipitate of albumin which crystallize more or less rapidly. The process is accelerated b inoculation with a crystal of egg albumin if availab and letting the mixture stand in the ice-box wit occasional stirring.

If the eggs were very fresh and the conditions c precipitation just right, crystallization may be complet within a few hours, but several days may be require

In case microscopic examination fails to reveal crystals, aliquot portions of the mixture are removed, one of which is treated with a small measured amount of acid, and another with alkali in order to find more favorable conditions for crystallization if possible. If these are found, the acidity of the entire mixture is adjusted accordingly, otherwise it is better to begin again.

Theoretically, the precipitation of a protein, which is built up of amino-acids and functions as a typical amphoteric electrolyte, should best be accomplished at the isoelectric point, that is, at the hydrogen ion concentration at which the acidic and basic functions of the protein are equal and at a minimum.[9] According to Soerensen, the iso-electric point of egg albumin is at pH 4.8,[10] and while precipitation would undoubtedly be most complete at this point, it is not necessarily the most favorable for crystallization, as the egg albumin crystals are not isoelectric protein, but a compound of this with the sulfate ion, and perhaps the ammonium ion as well.[11]

The mixture should be allowed to stand at least 24 hours in the cold after all amorphous material has become crystalline, in order to ensure a maximum yield. A little toluene may also be added to retard putrefaction.

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Recrystallization.

The crystals are finally filtered or centrifuged off, drained as well as possible from mother-liquor, and

[9] For a full discussion of the consequences of this viewpoint see Loeb, *Proteins and the Theory of Colloidal Behavior*, McGraw-Hill, 1922; Michaelis, *Wasserstoffionen-konzentration*, Berlin, 1914.

[10] It is assumed that the student is familiar with the meaning of this expression. If not, he is referred to W. M. Clark's book, *The Determination of Hydrogen Ions*, Williams and Wilkins, 1920.

[11] This point is fully discussed by Soerensen.

dissolved in as small a volume of water as possibl The amount required is surprisingly small, so tha only a little is added at a time to the crystals, wit constant stirring, and addition of water is stoppe before the solution is entirely clear It will be foun that a small portion of the albumin is usually "de natured," or rendered insoluble by the crystallizatio process, and this must be filtered off The pH of th slightly opalescent filtrate should then be adjusted t 5.2 to 5.4[12] Success in recrystallization of the albumi depends entirely on the hydrogen ion concentratio and if the reaction is too acid a gelatinous mass pr cipitates from which it is impossible to obtain crystal If the reaction is too alkaline, on the other hand, ver little of the albumin comes out

After the acidity of the concentrated albumin solu tion has been adjusted, saturated ammonium sulfa solution is stirred in, drop by drop, until enough albu min remains precipitated to make the solution milk in appearance Again the amorphous albumin gradu ally changes over into the crystalline form, and th precipitate increases in amount when the solution kept in the cold and stirred from time to time It best, at this point also, to have a little toluene presen

Three recrystallizations are sufficient to remove a but traces of the amorphous "conalbumin" and oth

[12] Young, *Proc Roy Soc* [B] 93, 15 (1921-2) gives 4 but good crystallization could not be obtained at this pH. is possible, however, that eggs from different sources va so that the student may have to determine the optimum con tions for himself.

A standard of pH 5 2 may be prepared by mixing 4.2 of 0.2 *N* acetic acid with 15 8 cc. of 0 2 *N* sodium aceta while the 5.4 standard contains 2 9 cc. of the acid to 1 cc of sodium acetate solution Bromo-cresol purple may used as indicator The range 5.2 to 5.4 was determined wit out correcting for the protein or salt error. For details pH determinations the student is referred to Clark's book.

impurities originally present, but each time a small amount of the crystalline albumin is usually transformed into an insoluble modification and must be removed. The crystals are stable only in the presence of the mother-liquor from which they are deposited, but an aqueous solution free from salts may be obtained by dialysis against water in the cold with the aid of a parchment or collodion membrane. Excellent collodion membranes for the dialysis of protein solutions may be prepared with the aid of one of the mixtures suggested by Eggerth,[18] e g., 7 g of "Parlodion" to 60 cc. of ether, 30 cc. of alcohol, and 10 cc. of glacial acetic acid. This mixture yields very tough membranes which are easily removed, after the addition of water, from the vessel in which they are formed. If a concentrated salt-free albumin solution is desired, the bag may be prepared in a 50 cc. test-tube, loosely filled with the albumin solution, and clamped at the open end with a rubber-faced screw pinchcock.[14] The pressure developed when the solution is dialyzed in this way prevents undue dilution. Complete removal of the salts may be tested for in the dialysis water by the Nessler method At least 4 or 5 daily changes of water are necessary before the test becomes negative In winter the dialysis may be completed more rapidly by immersing the bag in running tap water and finishing after 48 hours with one or two changes of distilled water.

$[\alpha]_D^{20}$ of a 10 per cent aqueous solution of pure crystalline egg albumin is about $-30.5°$.

[18] Eggerth, *J Biol. Chem.* 48, 203 (1921).

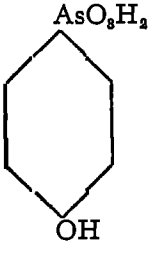
[14] This method of dialysis under pressure was suggested by Adair, Barcroft, and Bock, *J Physiol* 55, 332 (1921).

PrepBooks

IX. PREPARATION AND REACTIONS OF ORGANOMETALLIC COMPOUNDS.

(*See also p. 29*)

A. The Direct Arsenation of Phenol. *p*-Hydroxyphenylarsonic Acid.



Just as phenol can be nitrated or sulfonated, it is also possible to introduce the arsonic acid residue into the molecule by treatment of phenol with arsenic acid The following conditions have been found very satisfactory on a laboratory scale: [1]

Arsenation.

480 g. of 80 per cent aqueous arsenic acid are boiled in an open flask, allowing the water to escape until the temperature reaches 150°. 200 g. of molten phenol are then quickly poured in and an air-condenser is attached, after which the flask is placed in an oil bath kept at 155–60°. The contents of the flask

[1] Ger. pat 205,616, Jacobs and Heidelberger, *J. Am. Chem Soc.* 41, 1440 (1917)

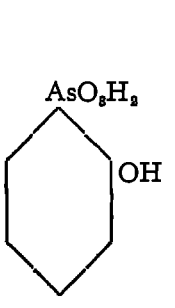
boil steadily (a few fragments of porous tile should of course be added), rendering mechanical stirring unnecessary. The air condenser (a long glass tube of about 1½ cm. bore) acts as an efficient reflux, preventing undue loss of phenol. The mixture is boiled for 7 hours, taking care, of course, that it does not boil so hard as to escape from the top of the condenser. Initially two layers are formed, but the mixture finally becomes homogeneous and darkens somewhat.

Purification.

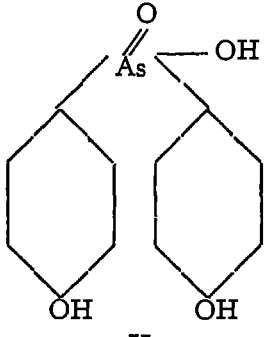
It is diluted with about 2 liters of water, resulting in the precipitation of a small amount of tar, and, without filtering, treated, with vigorous stirring, with a hot, strong solution of barium hydroxide until just neutral to litmus. It is important to avoid an unnecessary excess of barium hydroxide, for although the neutral barium salt of *p*-hydroxyphenylarsonic acid is soluble under these conditions, an excess may lead to the formation of basic salts which would be precipitated with the barium arsenate. The clear, almost colorless filtrate is then treated with just enough sulphuric acid to remove the barium ion present, a preliminary heating greatly facilitating the subsequent filtration of the barium sulfate. The precipitation should be followed during the addition of the sulfuric acid by tests on filtered samples, and it is a simple matter to strike the point at which the filtrate no longer reacts for either barium or sulfate ion.

The filtrate is next concentrated to about one liter, preferably *in vacuo*, and the further manipulations will depend on whether the student will proceed to the synthesis of "salvarsan (arsphenamine)," in which case two-thirds of the solution should be worked up for the sodium salt according to method 2, or whether he will stop at the isolation of the *p*-hydroxyphenylarsonic acid,

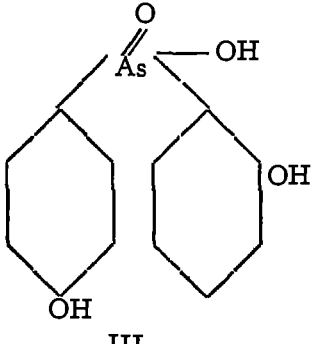
in which case method 1 alone should be followed. Instead of going over the salvarsan synthesis the student may also isolate some of the by-products of the arsenation of phenol, in which case two-thirds of the solution should also be worked up by method 2. Among the by-products are *o*-hydroxyphenylarsonic acid (I), *p*, *p'*-dihydroxydiphenylarsonic acid (II), and *o*, *p'* (?)-dihydroxydiphenylarsonic acid (III),



I.



II.



III.

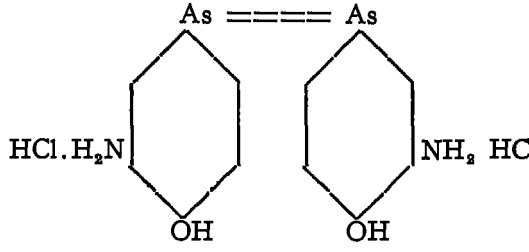
and a full theoretical discussion of the subject and directions for isolating the by-products will be found in the original article referred to above.

Isolation.

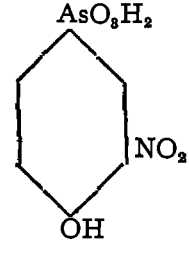
1. The solution (or one-third of it if the alternatives given above are followed) is further concentrated *in vacuo* to a thick syrup consisting of a mixture of free arsonic acids. This is dissolved in about 3 volumes of hot glacial acetic acid, yielding a faintly colored solution which gradually sets to a thick paste of colorless crystals of *p*-hydroxyphenylarsonic acid on chilling and rubbing. After standing 24 hours in the ice-box the acid is filtered off and washed with small portions of cold glacial acetic acid. The yield from 200 g. of phenol averages 40 g, or considerably less than if the acid is isolated as the sodium salt. The free acid melts at 170–3°, with preliminary softening.

2 Two-thirds of the concentrated solution are neutralized to litmus with sodium hydroxide and the solution concentrated further *in vacuo* until partial crystallization of the sodium *p*-hydroxyphenylarsonate occurs The mixture is then heated on the water bath until the salt redissolves, with the addition of a very small amount of hot water if necessary, and then treated while still hot with several volumes of alcohol until a slight permanent turbidity is observed. The sodium salt quickly separates, and after several hours of thorough chilling in a freezing mixture it is filtered off and washed with small portions of chilled 85 per cent alcohol. The yield from two-thirds of the original solution should be about 80 g., and if the manipulations have been carefully carried out the salt is pure, giving no test for barium, arsenate, or sulfate ions.

B. Synthesis of Salvarsan (Arsphenamine), 3,3'-Diamino-4,4'-dihydroxyarsenobenzene Dihydrochloride.



1. 3-Nitro-4-hydroxyphenylarsonic Acid,[2]



65 g. of carefully powdered and dried sodium *p*-hydroxyphenylarsonate are shaken into 230 cc. of concentrated sulfuric acid kept at 0°, avoiding the formation of difficultly soluble lumps by scattering the powder as well as possible. After being stirred mechanically until all is dissolved, the solution is immersed in a freezing mixture and a chilled mixture of 20 cc each of concentrated nitric and sulfuric acid dripped in at a rate such that the temperature of the well-stirred mix-

[2] Cf Ger pat. 224,953.

ture varies between —5° and 0° When the main reaction is over the vessel is removed from the freezing mixture and the stirring continued until the temperature reaches 10°. The solution is then poured into 700 g. of ice and water, stirred well, and allowed to stand over night in the ice-box The bright yellow arsonic acid is filtered off, washed with small portions of ice-cold water, and dried. The yield should be about 38 g

2. **Reduction of 3-Nitro-4-hydroxyphenylarsonic acid to 3,3′-Diamino-4,4′-dihydroxyarsenobenzene.**

In the reduction of an arsonic acid, the end product depends largely on the reducing agent used. For instance, sulfur dioxide and hydriodic acid together convert the arsonic radical into the arsinoxide group, —As = O, while stronger reducing agents such as hypophosphorous acid (also in the presence of hydriodic acid) or sodium hyposulfite (hydrosulfite) effect reduction to a still lower stage, that of the arseno- compounds —As As—, in which two molecules of the arsonic acid are linked through the arsenic atoms after reduction. Zinc dust and hydrochloric acid reduce arsonic acids even more powerfully, giving arsines, $RAsH_2$.

When the reduction of a nitrophenylarsonic acid is in question the state of affairs is still more complicated, for it becomes possible to reduce either the nitro- or the arsonic-acid group without affecting the other, or to reduce both simultaneously. For example, ferrous sulfate and alkali give aminophenylarsonic acids, while sulfur dioxide and hydriodic acid together permit the preparation of certain nitrophenylarsinoxides. Sodium hyposulfite (hydrosulfite) in excess, on the other hand, reduces the nitro- to the amino- group, with simultaneous formation of arseno compounds. This reagent was used

by Ehrlich and Bertheim [3] for the direct preparation of "Salvarsan," and while other methods are said to yield a product of lower toxicity,[4] the method is given here as well for its historical interest as because it avoids the isolation of an intermediate product and gives satisfactory yields when carefully carried out.

Reduction.

A solution of 25.7 g. of magnesium chloride crystals [5] in 650 cc. of water is poured into a 1.5 liter beaker suspended in a water bath. A thermometer and mechanical stirrer are adjusted in the beaker, after which 148 g. of commercial sodium hyposulfite (hydrosulfite) (80 per cent) are stirred in, avoiding the formation of lumps, and followed immediately by a cold solution of 9.9 g. of 3-nitro-4-hydroxyphenylarsonic acid (0.038 mols.) in 225 cc. of water and 68 cc. of 10 *N* sodium hydroxide. The water bath is then heated until a temperature of 55–60° is maintained within the beaker. A yellowish, amorphous (contrary to Ehrlich and Bertheim) precipitate soon begins to separate, and gradually increases in amount as the reaction proceeds. After one and a half to two hours' stirring a filtered test portion should remain clear or give only a slight turbidity on heating to the boiling point. The mixture is then filtered on a Buchner funnel, washed well with water, and pressed down thoroughly.

Purification.

The crude, very impure, water-insoluble, easily oxidizable arseno-compound is then converted into its dihydrochloride (Salvarsan, Arsphenamine). The

[3] Ehrlich and Bertheim, *Ber.* 45, 756 (1912).
[4] Cf. Christiansen, *J. Am. Chem. Soc.* 42, 2402 (1920).
[5] The presence of this salt in some way prevents the formation of undesirable by-products.

calculated amount of strong methyl alcoholic hydrochloric acid (0.038 mols HCl) is added to 85 cc. of methyl alcohol and the crude product stirred with this until as much as possible dissolves. The insoluble material is collected with the aid of decolorizing carbon and the mixture filtered. The filtrate is added, with stirring, to one liter of chilled ether (Ehrlich and Bertheim) or acetone and the amorphous (contrary to Ehrlich and Bertheim) yellow precipitate is washed with acetone or ether and dried *in vacuo* over sulfuric acid and paraffin, after which it still retains solvent corresponding roughly to one molecule of methyl alcohol. The yield should be about 80 per cent of the theory, or 7 g.

The entire reduction and purification should be carried out in as short a time as possible, and with the minimum exposure of the product to the air, for not only is the arsenic of the arseno group easily oxidizable, but the *ortho*-aminophenol grouping in addition makes the compound extremely sensitive.

The product obtained in this way is not an absolutely pure chemical individual, and usually contains an appreciable amount of sulfur (2 to 3 per cent), at least a portion of which appears to be present as a sulfonic acid.[6] The purity of the product may be controlled by an arsenic determination, or better, by titration with iodine after dissolving in water and acidifying slightly with hydrochloric acid, one molecule of the arseno compound being oxidized to two of 3-amino-4-hydroxyphenylarsonic acid under these conditions according to the equation:

$$RAs:AsR + 4I_2 + 6H_2O \longrightarrow 2RAsO_3H_2 + 8HI$$

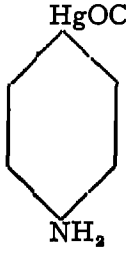
If the arseno compound is to be preserved it must be kept in vacuum ampoules, for it undergoes oxida-

[6] King, *J. Chem. Soc.* 119, 1416 (1921).

tion even in the dry state and as the hydrochloride. Practically, the resultant alteration involves serious dangers, as the toxicity of the drug is thereby greatly increased

C. Mercuric Compounds of Aniline.

1. *p*-Aminophenylmercuric Acetate,



The "mercuration" of aromatic compounds is of sufficiently general applicability to be classed with such processes as bromination, nitration, and sulfonation, and is particularly easily carried out in the case of phenols and amines.

To a solution of 63.6 g. of pulverized mercuric acetate in 320 cc. of water containing a few drops of acetic acid to suppress hydrolysis, add 37.2 g. of aniline, shake until dissolved, and filter rapidly if the solution is not clear. Entrance of the mercury into the nucleus takes place with extreme ease, giving mainly the *p*- compound, which soon begins to crystallize. Since deposition of the isomeric *o*- acetate, which is formed in relatively small amount, also takes place after several hours, the crystals are filtered off after three hours, thus avoiding contamination with the mixture of the

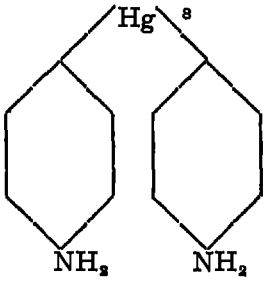
[7] Cf. Dimroth, *Ber.* 35. 2038 (1902).

two compounds which would separate later A small portion of the *p*-aminophenylmercuric acetate should be recrystallized from chloroform for analysis, the melting point being 166–7°. The remainder is sufficiently pure for the preparation of *p*-mercuri-*bis*-aniline.

The isomeric *o*-aminophenylmercuric acetate is difficult to separate from the larger amount of the *p*- compound which would separate with it if the filtrate from the initial crop of the *p*- acetate were allowed to stand. Dimroth found, however, that there was a considerable difference in the solubilities of the chlorides, so the filtrate is treated with an excess of sodium chloride solution, precipitating *p*-aminophenylmercuric chloride in the amorphous state, while the *o*-amino chloride separates in crystalline form. The mixture is sucked off, dried, and the *o*- derivative extracted with not too much warm alcohol, separating as leaflets after the solution is chilled and allowed to stand The amorphous residue of *p*- chloride may be boiled with alcohol or benzene, and separates from the cold solution as leaflets melting at 188°, with decomposition.

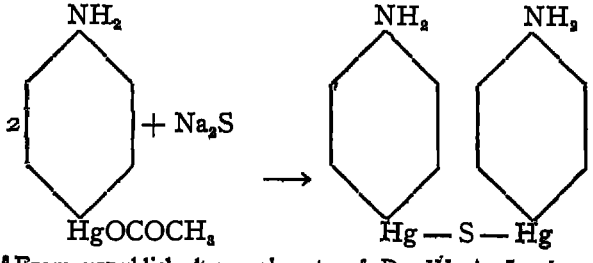
In all of these compounds the mercury retains its salt-forming character, and mercuric ions are split off with relative ease. For instance, boiling the salts with sodium or ammonium sulfide solution results in the deposition of mercuric sulfide, while alkaline stannous chloride solution immediately gives a dark gray precipitate of mercury. The latter test is, in fact, a very convenient one for identifying mercury compounds of this, the so-called "half complex" class, in which one valence of the mercury retains its salt-forming inorganic character, as distinguished from the "full complex" class, in which the mercury is fully bound between two organic radicals, and is more completely masked. The preparation of a typical member of the "full complex" group is given below.

2. ***p*-Mercuri-*bis*-aniline,**

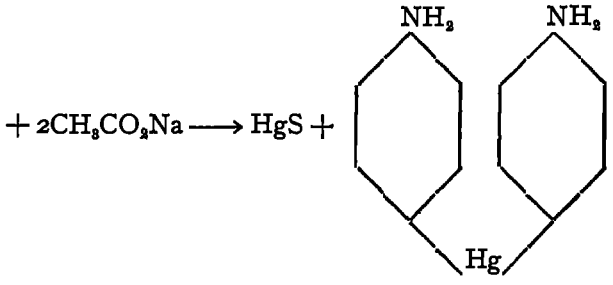


18 g. of *p*-aminophenylmercuric acetate are finely pulverized, suspended in 200 cc. of warm water, stirred mechanically on the water bath, and treated with aqueous ammonia until the substance is entirely dissolved. Just as inorganic mercuric salts form complex ions with ammonia, so do the aminophenylmercuric salts and in this case the ammonia compound is easily soluble.

A solution of 14 g. (1.1 equivalents) of crystalline sodium sulfide in a little water is next added, and the stirring and heating continued for two and one-half hours. Mercuric sulfide and crystalline *p*-mercuri-*bis*-aniline are precipitated apparently according to the scheme.



[a] From unpublished experiments of Dr. W. A. Jacobs and the writer.



The precipitate is then filtered from the hot solution, washed with a little hot water, sucked dry, and extracted with pyridine on the water bath, as this solvent dissolves much larger quantities of the mercuri compound than chloroform, which is usually proposed. After concentrating to small bulk *in vacuo* the solution is carefully diluted with hot water and rubbed, whereupon the *bis*-compound gradually separates as glistening needles which melt at 174° with decomposition The yield should be 6–7 g. In contradistinction to the starting material, the *bis*-compound does not blacken when treated in the cold with alkaline stannous chloride solution.

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